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CYTOLOGICAL STUDIES IN THE BETULACEAE

I. BETULA[1]

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(WITH PLATES XI, XII)

Introduction

It is now well known and widely recognized that many of the polymorphic groups of plants owe their condition to the readiness with which their species cross. An outstanding and interesting instance is the production, by European monographers (ZAHN 51), of five large volumes devoted entirely to the description of species and variants in *Hieracium*, and the collateral cytological and genetical investigations of ROSENBERG (36, 38) and OSTENFELD (32, 33, 34) which show that *Hieracium* is composed largely of heterozygous forms.

Taxonomic works list several hybrids in both *Corylus* and *Alnus*, and at least fifteen recognized hybrids in *Betula*. Since the group is well known to be polymorphic, it seemed that this very condition might be due to some unrecognized hybridity. This study apparently substantiates such a view.

The birches grow exclusively in the cooler regions of the northern hemisphere, attaining higher latitudes than any other deciduous tree except the mountain ash. From their circumpolar range they radiate out from the north into Europe, Asia, and North America in considerable numbers of parallel forms, which fall into somewhat

[1] Contribution from the Laboratories of Plant Morphology, Harvard University.

definite geographic entities. They are baffling in their variability and very difficult to classify. REGEL, the greatest student of the genus, never used the same treatment twice in his monographic studies. First a group comprised one species, then several species, and finally all phases were considered as species.

Europeans recognize two species, *Betula pubescens* Ehrh. (*B. alba* L.) and *B. verrucosa* Ehrh. (*B. pendula* Roth.). In northern Scandinavia, Iceland, and Greenland are many dwarf forms which are treated as variants of the native species. In Labrador and on Mount Washington and Mount Katahdin are dwarf birches which are called variants of American species. They, however, are not distinguishable from the northern European forms. FERNALD (12) writes of this inconstancy of specific lines:

> The dwarf birches like the canoe birches present such tendencies to intergradation that it is difficult to draw clear specific lines between them..... In conclusion, it should be emphasized that the specific lines in *Betula*, *Alnus*, *Quercus*, and *Salix* are often too vague. It is quite possible to trace by a series of specimens a direct connection between the dwarf *B. nana* and the tall *B. alba*. Thus *B. nana* in its larger development is separated with difficulty from the Scandinavian *B. alpestris*. This shrub in turn is quite like glabrate states of the American *B. pumila*, which, through its variety *glandulifera*, passes to *B. glandulosa*, the larger developments of which pass in the Cascade Mountains to *B. microphylla*, and in the Saskatchewan region to *B. alba* var. *minor*. The latter shrub is often inseparable on the New England mountains from *B. papyrifera* var. *cordifolia*, which on the lower slopes becomes a large tree and passes gradually to the broad-leaved form figured by MICHAUX as *B. papyracea*. A very similar series is readily made to include *B. pendula* and *B. humilis*. But since it is obviously impracticable to regard all these forms as one species, it seems wiser to recognize the more marked centers of variation as species which are admitted to pass by exceptional tendencies to other forms ordinarily distinguished by marked characteristics.

When the members of a large group of plants exhibit such diversity and consequent uncertainty of specific lines that call for frequent revisions, it affords a fertile field for investigations concerning evolutionary tendencies. It has long been known that a number of birch species hybridize more or less freely in nature and under cultivation. JACK (20), ROSENDAHL (39), FERNALD (13), and HELMS and JORGENSEN (19) have contributed important studies on hybrid birches. WINKLER (49) describes eight natural *Betula* hy-

brids and lists six others. They are: *pumila*×*lenta*, *pubescens*×*nana*, *verrucosa*×*humilis*, *pubescens*×*humilis*, *pubescens*×*verrucosa*, *nana*×*verrucosa*, *populifolia*×*papyrifera*, *papyrifera*×*pumila* var. *glandulifera*, *lutea*×*pumila* var. *glandulifera*, *davurica*×*lenta*, *pumila*×*nana*, *pumila*×*glandulosa*, *verrucosa*×*papyrifera*, and *coerulea-grandis*×*populifolia*. With these conditions obtaining, it seemed that a cytological investigation of the reduction division in species of *Betula* might disclose one of the reasons for their marked variability. This supposition has proved correct, and some interesting results are at hand.

Materials and methods

Material for this study was gathered from labeled specimens in the Arnold Arboretum, where have been brought together a representative collection of the species of *Betula*. Staminate catkins were collected in the early afternoon on warm sunshiny days during the first half of September. The catkins were slit with a sharp razor and immediately put in Carnoy's fluid. By the use of an exhaust pump the air was quickly withdrawn from the tissues to insure rapid fixation. After 24 hours in the fixing fluid the catkins were placed in 95 per cent alcohol, where they remained until the imbedding process.

Experiment proved demineralizing and bleaching to be unnecessary. The material was dehydrated and imbedded in nitrocellulose, which is proving much superior for cytological work than is paraffin. The method employed is that described by JEFFREY (21). Small catkins were treated by the mass method, as described in JEFFREY'S (22) report of meiosis in *Drosophila*, wherein numbers of small objects for sectioning are fixed close together on cardboards, stuck with glycerin jelly, imbedded, and sections of a great number of small objects taken with one stroke of the microtome knife.

Sections were cut with a Jung-Thoma sliding microtome about 10 μ in thickness. Thinner sections were not satisfactory because many mother cells were cut, thus making them useless for study. Haidenhain's iron haematoxylin gave the best staining results. COLE'S (9) rapid iron haematoxylin technique was also quite satisfactory.

The study and drawing of material were done with a Bausch and

Lomb microscope equipped with a 130 Abbé condenser, a 1.5 mm. 130° Zeiss apochromatic objective, and an 8 power Leitz (periplan) compensating ocular. Strong light was obtained from an 150 watt bulb light. Drawings were outlined from typical stages of pollen mother cells during the reduction divisions with the aid of a camera lucida. All P.M.C's. have a thick callose coat, as shown in figs. 33–44. Magnification is the size increase as it appears in print after reduction from the larger original drawings. In the cytological descriptions X indicates the haploid number of chromosomes and $2X$ the diploid.

General microsporogenesis

The meiotic divisions in a normal plant or animal progress quite regularly. In diakinesis the normal homology between paternal and maternal chromosomes brings them together as bivalents. At heterotypic metaphase the chromosomes are arranged definitely, as in fig. 3. At heterotypic anaphase the chromosomes progress in an orderly manner to the poles, as shown in fig. 41. The homeotypic division is not unlike an ordinary mitotic division.

There are occasions when the meiotic phenomena are characterized by abnormalities rather than by the normal division just described. These irregularities in general are due to the disharmony of the paternal and maternal chromosomes when they have their origin in individuals of different species. In other words, irregularities of the meiotic phases are intimately connected with hybridization. An examination of figs. 33–40 makes the scope of these abnormalities quite apparent, and shows the various stages of meiosis in the hybrid *Betula jackii* (*B. lenta*×*pumila*), which will be discussed in detail later. When the parents of the hybrid differ in their chromosome numbers the meiosis may show marked abnormalities, but if the parents have the same chromosome number, this is not necessarily so; the divisions may be marked by regularity. This brings out the important fact that all hybrids do not exhibit irregular meioses. Also, if they can produce viable seed and proceed to the second filial generation, they may eliminate the causes of abnormalities and become full fledged species which breed true. On the other hand, they may be so sterile that the seed is formed from nucellar budding. These asexually reproducing clones are of course very

stable genetically, and are considered to be good species; but an examination of the reduction division shows that they are heterozygous. The Caninae section of *Rosa* shows this condition very markedly.

Cytology of Betula species

DIPLOID SPECIES

Subsection Costatae

Betula lenta L., X-14 (meiosis normal).—Fig. 43 shows a heterotypic metaphase plate showing the fourteen chromosomes, which stand unusually far apart, compared with the condition obtaining in other species. Occasionally, however, two of the gemini stand so close together that they appear to be fused into one large mass, as happens so typically in *Corylus*. Fig. 44 shows the initiation of the early anaphase of the first division. Cytomyxis is marked and very common during the prophase, the cytoplasmic strands usually contracting by the time the metaphase is instituted. They are again apparent during interkinesis.

B. nigra L., X-14 (meiosis normal).—The phases of the reduction division compare favorably with those of the preceding species. Fig. 27 shows the heterotypic metaphase plate with fourteen chromosomes. The early homeotypic metaphase seems to be characterized by tardiness of the chromosomes in their action on the spindle. During the prophase there is much cytomyxis between the P.M.C.'s. The reduction division takes place two weeks later than in most species (latter part of September).

B. schmidtii Regel, X-14 (meiosis very abnormal).—When the gemini are forming there is such an incompatibility between some of the chromosomes that they do not form synaptic mates. These then remain as univalents, and cause abnormalities which are known to be intimately connected with plants of heterozygous origin. Fig. 23 illustrates the phase corresponding to the heterotypic metaphase. Ten pairs of chromosomes have united, forming gemini, the remaining eight existing as univalents. Not only the latter but also the bivalents are behaving queerly on the spindle. One of the single chromosomes has left the spindle and is reposing in the cytoplasm; other cells show equally involved conditions. Sometimes there is a

fusion of a few of the chromosomes into a single large mass. Occasionally the heterotypic metaphase appears to be quite normal and the polar view shows fourteen chromosomes (fig. 25). This is an important trait of heterozygotic plants which perhaps is not commonly realized. Along with the abnormalities occur some few normalities, which are capable of producing morphologically good pollen grains. This of course is the reason that the pollen is not entirely sterile in every cross. Instead of the formation of a normal tetrad of nuclei, a polycaric mother cell is often formed with five, six, and seven nuclei. Fig. 24 shows such a cell. This polycary may be formed, in part at least, by the migration of whole nuclei from one cell across cytomyctic connections into adjoining cells. The writer has seen unmistakable evidence of this. The pollen appears to be normal. In view of the conditions obtaining in the reduction division of the P. M.C.'s in this species, it is necessary to consider that it may be of hybrid origin.

Subsection Albae

×*B. coerulea* Blanchard (*B. coerulea-grandis*×*populifolia*).—In Gray's manual the following appears under *B. pendula* Roth: "A polymorphous boreal species, of which the New England phase has recently been designated as *B. coerulea* Blanchard (blue birch)." Both Fernald (13) and Rehder (35a) consider this small tree to be a hybrid between *B. coerulea-grandis* and *B. populifolia*. Sargent's (41) suggestion, "Perhaps with its variety best considered a natural hybrid between *B. papyrifera* and *B. populifolia*" is seen to be quite impossible, since *B. papyrifera* is a pentaploid species and *B. populifolia* is diploid. *B. coerulea* is also diploid. This species is then best considered a natural hybrid between *B. coerulea-grandis* and *B. populifolia*, both of which have fourteen as the haploid number of chromosomes.

X-14 (meiosis normal).—Fig. 28 shows the heterotypic metaphase plate with fourteen chromosomes. The reduction division is quite regular in its behavior, with the exception that occasionally one or two of the bivalent chromosomes are tardy in their actions on the spindle, and sometimes do not pull apart until the anaphase is well developed. The pollen is normal. The mother cells exhibit

marked cytomyxis and chromatolysis during the prophases. Sometimes the greater part of a nucleus is seen to have crossed the cytoplasmic bridge and is crowding the nucleus of the cell over to the opposite side.

B. coerulea-grandis Blanchard, X-14 (meiosis normal).—Fig. 29 shows the heterotypic metaphase plate with fourteen chromosomes. During the first division the chromosomes are frequently seen to be split for the homeotypic division. Cytomyxis and chromatolysis are marked during the prophases. In one anther a peculiar condition is at hand. Ten mother cells in the prophase have completely fused together, all but the outer cell walls having been dissolved, forming a large continuous mass of protoplasm with chromatin distributed throughout.

B. fontinalis var. *piperi* Sarg., X-14 (meiosis normal).—The chromosome count was made at diakinesis and interkinesis. No phases of the heterotypic division were seen. Both prophases and interkineses were accompanied by marked cytomyxis and chromatolysis. One interkinetic cell had received a whole nucleus from a neighboring cell and the cytoplasmic bridge had practically disappeared. This species has been considered to be a phase of *B. papyrifera*, but that apparently is not the true relation of the plant, because *B. papyrifera* is pentaploid while this appears to be diploid.

B. japonica Sieb., X-14 (meiosis normal).—Fig. 13 shows the heterotypic metaphase plate with fourteen large chromosomes. During the anaphase of this division there appeared many small black bodies in the cytoplasm. These are minute and are not to be confused with chromosomes.

B. japonica var. *mandshurica* (Regel) H. Winkl.—This is apparently a hybrid, since it has forty-two chromosomes as the reduced number rather than the fourteen of the true species. It is a diploid-hexaploid cross, and is treated under the heading "Hybrid tetraploid."

B. pendula Roth., *B. verrucosa* Ehrh.—Although the first name is some three years older, many botanists have called this species by the latter name.

X-14 (meiosis abnormal).—Fig. 30 illustrates a diakinesis with thirteen gemini and two univalents. Many cells in this phase of de-

velopment have been examined. The affinity of the supposedly homologous chromosomes is variable: some cells have eleven bivalents and six univalents; others have twelve bivalents and four univalents; while others are normal with fourteen bivalents. The mother cells with the univalent chromosomes show irregularities during meiosis. The number of lagging chromosomes is directly due to the non-formation of gemini during the prophase. Several anthers contained cells in the homeotypic division which were behaving very queerly. The chromosome masses at the spindle poles were breaking up and migrating across cytomyctic bridges into neighboring cells. The quartet of pollen grains is normally formed, but the cells often contain extruded chromosomal bodies. The tapetal cells (sporophytic tissue), while containing twice the number of chromosomes of the meiotic cells, are entirely normal in nuclear division. Fig. 26 shows a group of P.M.C.'s undergoing extreme chromatolysis during the prophase. In three of the cells the nuclei have completely resolved into streaming black strands of chromatin, which sometimes stretch from one cell right across another to a third.

B. pendula has so many phases that it seems quite possible that the specimen on Bussey Hill in the Arnold Arboretum, which was obtained from MEEHAN in Pennsylvania, may be a natural hybrid. This idea is strengthened by the fact that HELMS and JORGENSEN (19) have worked out the cytology of *B. verrucosa*, *B. pubescens*, and many natural hybrids between the two species, all of which are growing in a large bog in northern Sweden. Their results show the meiotic divisions of *B. verrucosa* (*B. pendula*) to be quite regular, with fourteen chromosomes. Thus the cytological evidence at hand indicates that the diversity of the species may be due to its ready crossing with some other diploid species.

B. populifolia Marsh., X-14 (meiosis normal).—Figs. 1 and 2 portray the heterotypic metaphase spindle from side and polar views respectively. Occasionally, during the first division, a bivalent retains its position at the plate until late anaphase, when it separates and its halves go to their respective nuclei. Small dark bodies are seen in the cytoplasm at various stages. They are not chromosomal in nature. Cytomyxis was noted at interkinesis.

Subsection Acuminatae Regel

B. maximowicziana Regel, X-14 (meiosis normal).—Fig. 31 shows the heterotypic metaphase plate with fourteen chromosomes. Cytomyxis occurs at prophase.

TRIPLOID HYBRIDS

Subsection Costatae×Nanae

×*B. jackii* Schneid. (*B. lenta*×*pumila;* diploid×tetraploid).—Jack (20) describes the origin of this hybrid as follows:

> From a vigorous plant of this dwarf birch (*B. pumila*) which was growing about a hundred paces to the east of a number of healthy, well-grown trees of *Betula lenta*, a large quantity of seed was collected in the autumn of 1887. This was sown in January 1888 and resulted in several thousand seedling plants. As these grew, a large number were disposed of or destroyed. Out of several hundred plants which have been kept to maturity, half a dozen individuals show distinct characters, indicating hybridity with *B. lenta*, the only species growing in the vicinity and the pollen of which would be directly blown to the seed plant by the prevailing westerly winds.

Jack also points out the characters of the hybrid, illustrating them along with those of the parents, and showing that they are intermediate in all ways. He continues:

> The present instance seems to show how freely the birches may hybridize with each other when two or more species are growing near together and flowering at about the same time. But when growing naturally, although many ovules might be fertilized by pollen from another species, it would be a rare case that one of the perfected seeds should develop into a mature plant as not one seed in tens of thousands gets a chance to grow. On the other hand, under conditions of artificial propagation and cultivation, a large proportion of the seeds sown are expected to develop into plants, and it is under these circumstances that the fact of the frequency of hybridity becomes apparent. It is made especially clear when it occurs among such dissimilar species as *Betula pumila* and *B. lenta*, where the intermediates are so distinct that hasty students might be led to give them new specific names. It may be worth mentioning that Mr. C. E. Faxon and other botanists have found in various parts of New England what appear to be undoubted hybrids between *B. papyrifera* and *B. populifolia*.

The cytological investigation of the reduction division in *B. jackii* proves to be a distinct correlation with this report.

X-21 (meiosis very abnormal).—The diakinesis has not been a satisfactory stage for study, on account of the number of chromo-

somes and the small size of the cells. Fig. 33 shows a diakinetic mother cell which appears to have seventeen univalents, twenty-three bivalents, and two trivalents, although each clump must be the product of but one chromosome, because there are just forty-two clumps and there are just forty-two chromosomes from the two parents. This is apparently one of the cells mentioned later in which no reduction takes place. Another cell shows but thirty-five of these clumps, of which seven are bivalents and the remaining twenty-eight univalents. Many of the latter are already split for the second division, however, and appear as if bivalent in nature. This stage of meiosis is typified by abnormality of gemini formation.

Fig. 34 shows the phase just previous to the heterotypic metaphase, with bivalent and univalent chromosomes scattered all along the spindle. Later on the bivalents arrive at the metaphase plate, while the univalents still lag on the spindle; and sometimes, just as the bivalents are breaking, the univalents draw in, so that, if this stage be viewed from one of the poles, a count can be made of the plate. These counts vary extremely according to the number of gemini formed during the prophases. The most usual condition is that which results from the pairing of the fourteen chromosomes from the pollen parent with fourteen from the seed parent, the other fourteen from the latter remaining univalent. This gives a metaphase plate with twenty-eight (fig. 37) chromosomes, apparently half of them bivalent in nature. Occasionally two or four of the univalents pair, giving rise to one or two extra bivalents, thus making the count of the plate twenty-four or twenty-six. More often all fourteen bivalents are not formed, and the counts are thirty and over. The extreme of this tendency is no pairing of any of the chromosomes. This condition obtains frequently, and of course the cells involved experience no heterotypic division as no reduction takes place. Fig. 35 shows the plate of such a cell, showing clearly forty-two chromosomes. Two-thirds of them are large, apparently having come from the tetraploid parent (*B. pumila*), while the other third are smaller and have come from the diploid parent (*B. lenta*). Since there is no reduction, all of the chromosomes split, and the cell undergoes a mitotic division (homeotypic), producing two diploid nuclei, and subsequently a diad of diploid pollen grains. Fig. 38

shows the metaphase of this single division. The spindle is very broad, and there are no gemini.

Fig. 36 illustrates the anaphase of the first division of one of the heterotypic cells. Univalents are tardy in their movements, and some of the bivalents are undivided. The homeotypic division is likewise typified by lagging of the chromosomes, as shown in fig. 39. Single chromosomes are often extruded into the cytoplasm. Fig. 40 shows polyspory, rather than the normal tetrad, caused by the formation of extra pollen grains from the chromosomes which lagged on the spindles so long that they were not included in the polar nuclei when their nuclear membranes were formed. The pollen grains vary greatly in size, from microcytes to more or less normal-sized grains, and to large double-sized grains. The smaller and some of the larger ones degenerate, causing considerable sterility. ROSENDAHL (39) finds the pollen 25 per cent sterile.

Meiosis and pollen formation are typically normal in both the parents. Fig. 41 illustrates the heterotypic anaphase of the seed parent (*B. pumila*); fig. 42 shows the metaphase plate with twenty-eight large chromosomes. Fig. 43 illustrates the heterotypic metaphase plate of the pollen parent (*B. lenta*), showing fourteen smaller chromosomes. Fig. 44 is the early heterotypic anaphase of the same plant.

The contrast between the normal meioses of the parents and the very irregular meiosis of this hybrid comprises another very distinct proof of the close relation existing between hybridization and resultant meiotic irregularities. The apparent cause of the latter is the lack of homology between the chromosomes during gemini formation. Cytomyxis was frequently noted at prophase.

SUBSECTION ALBAE

B. verrucosa Ehrh. × *pubescens* Ehrh.—HELMS and JORGENSEN (19) are the authors of the taxonomy and cytology of this hybrid. *B. verrucosa* Ehrh. has fourteen gametophytic chromosomes, and meiosis is normal; *B. pubescens* Ehrh. has twenty-eight gametophytic chromosomes, and meiosis is normal. The hybrid between these two species is triploid, with forty-two sporophytic chromosomes. At diakinesis there are fourteen bivalents and fourteen univalents.

During the first and second divisions the univalents are very tardy in their movements on the spindle. The pollen grains show much variation in size and exhibit polycary. One pollen grain is shown with four nuclei. The pollen of the parents is quite normal. HELMS and JORGENSEN present a sketch map of the large bog in northern Sweden where the plants which they studied grew. The positions of the parents and hybrids of the F_1 and F_2 generations are plotted. Drawings of leaves, cone scales, and seeds, and excellent photographs of the individual trees clearly show the intermediate nature of the hybrids. Their paper is altogether a very convincing one.

TETRAPLOID SPECIES

SUBSECTION NANAE

B. pumila L., X-28 (meiosis normal).—Figs. 41 and 42 show the heterotypic anaphase and the metaphase plate respectively. Cytomyxis and chromatolysis occur during the prophase. In one anther several of the P.M.C's. had their nuclei sending fingerlike processes into neighboring cells. The chromosomes extended into the extruded portions of the nucleus over into the next cells, but they were never seen to have been extruded into the cytoplasm.

This tetraploid species is an accomplished hybridizer. It is one of the parents in *B. jackii*, *B. sandbergi* (*pumila* var. *glandulifera*×*papyrifera*), and *B. purpusii* (*pumila* var. *glandulifera*×*lutea*).

SUBSECTION ALBAE

B. papyrifera var. *cordifolia*. (Regel) Fernald, X-28 (meiosis slightly abnormal).—At diakinesis there is an occasional non-affinity between a few of the chromosomes. This is indicated by from one to six univalent chromosomes lagging on the spindle during the heterotypic division. Fig. 9 shows the metaphase plate of the first division with twenty-eight chromosomes. Many of the mother cells contain very broad spindles. A study of these cells showed them to contain double the number of chromosomes, which appeared to be about half size, suggesting that reduction has failed or that semi-heterotypic division (ROSENBERG **38**) has taken place. I have sought in vain for the restitution nucleus of this latter type of division. Counts of the metaphase plates of these cells show them to

contain fifty-six chromosomes (fig. 10). The mitotic division of this cell with the broad spindle results in the formation of a diad of diploid pollen grains. This is discussed under polyploidy.

Cytomyxis and chromosome migration take place frequently. Fig. 11 is a typical example of cells so involved. The chromosome complement is passing across cytoplasmic connections into nearby cells. If the migration is completed and the cells live, we have here one of the possible origins of polyploidy. This was seen in other species and is discussed later.

It is most interesting to note that this variety is tetraploid, while the true species is pentaploid with thirty-five chromosomes as the haploid complement. This is an impossible situation. In any case a difference in chromosome number is certainly adequate evidence for considering the two plants of specific rank. In this case, to be sure, the separation on gross morphological characters is not an easy one, especially in the aberrant forms. This indicates that cytology is coming to be of great importance in clarifying the haziness of the species concept.

B. pubescens Ehrh., X-28 (meiosis normal).—HELMS and JORGENSEN (19) are the authors of the cytology. This species hybridizes readily with the other European species, *B. verrucosa* (*B. pendula*).

HYBRID TETRAPLOID

SUBSECTION ALBAE × COSTATAE (DIPLOID × HEXAPLOID)

B. japonica var. *mandshurica* Winkl., 2X-56 (meiosis very abnormal).—*B. japonica*, one of the parents of this hybrid, has been noted previously to be very normal as regards meiosis. Fig. 13 shows the heterotypic metaphase plate with fourteen chromosomes.

The meiosis of var. *mandshurica* stands in marked contrast to that of *B. japonica*. The count of the metaphase plate of the first division is forty-two rather than fourteen. There are many univalent chromosomes in evidence during the reduction divisions. Fig. 15 shows a typical metaphase of the first division with these single chromosomes lagging on the spindle. Often these univalents get in to the equatorial plate just previous to the separation of the gemini, so that a chromosome count from the polar view is readily made.

Most of these plates show forty-two chromosomes, fourteen of which are consistently larger than the others (fig. 14). These latter are apparently from the *B. japonica* parent, and are bivalent in nature. The unpaired univalents surround these gemini, numbering twenty-eight. The count varies; sometimes all fourteen of the *japonica* chromosomes do not find mates, thus the count runs as high as forty-five. Then again, not only do the fourteen pair, but so do some of the smaller chromosomes among themselves; thus carrying the count down to thirty-nine. All counts from thirty-nine to forty-five have been made, forty-two being the commonest. Figs. 15 and 16 illustrate two typical phases in the heterotypic division. Large numbers of univalents lag on the spindle. Now a very significant phenomenon takes place. The bivalents and some of the univalents are at the poles of the heterotypic anaphase spindle and incite the formation of nuclear membranes as in the telophase; but so many of the univalents remain on the spindle that these membranes are unable to form through the spindle, and one continuous membrane is laid down around the complete nuclear figure. Between the two poles this membrane is constricted (fig. 47), giving the appearance of the nucleus pulling apart without the formation of a spindle. The nucleus later resumes the usual shape, and of course contains the diploid number of chromosomes. This large nucleus now passes through the second division with but one spindle, and results in the formation of a diad of diploid pollen grains. A large number of these diads appear in the anthers with the tetrads.

When this semiheterotypic division does not take place, the homeotypic division also has lagging chromosomes on the spindle. The mature pollen exhibits a wide range in size. Some of the grains are microcytes, others are larger and more normal, and the diploid grains from the diads are double-sized. A small proportion of the grains are defective. Cytomyxis and chromatolysis are both present to a marked degree during the prophases.

On account of the cytological evidence, this variety must be considered a hybrid between *B. japonica,* which it resembles very much, and some hexaploid species. *B. grossa* appears to be the only known hexaploid species growing in northeastern Asia, but a study of herbarium material produces very little evidence which would

indicate it to be one of the parents. Subsequent study may show *B. davurica*, here considered a dysploid hybrid with about forty-five gametophytic chromosomes, to be involved in the cross.

PENTAPLOID SPECIES

Subsection Albae

B. papyrifera Marsh., X-35 (meiosis almost normal).—Figs. 5 and 6 show the heterotypic metaphase from side and polar view, the latter clearly showing the thirty-five chromosomes. Most of the P.M.C's divide quite normally. Occasionally there are from one to six lagging univalents during the homeotypic division. Cytomyxis and chromatolysis are marked during the prophase. Many small dark bodies are noted in the cytoplasm. They are not to be considered chromosomal in nature.

This species has been considered part of the true *B. alba* L. In Europe *B. alba* L. is called *B. pubescens* Ehrh. Helms and Jorgensen find the latter to be tetraploid, with twenty-eight gametophytic chromosomes; so the supposed affinity is not real. Detailed cytological investigation may serve to help untangle the involved taxonomic conditions of the white birches.

HEXAPLOID SPECIES

Subsection Costatae

B. lutea Michx. f., X-42 (meiosis somewhat abnormal).—Diakinesis usually showed forty-two gemini. Occasionally univalents and trivalents were seen among the bivalents. Figs. 7 and 8 show the heterotypic spindle from side and polar views, the latter with forty-two chromosomes; some counts went as high as forty-nine. These were in cells where conjugation did not occur among all the homologous chromosomes. In such cells lagging univalents appeared on the spindles. Several cells were noted which, during the first division, extruded several chromosomes into the cytoplasm. These form microcytes which degenerate. Cytomyxis and chromatolysis are very marked, especially during prophase. Several cases have been noted where the diakinetic nucleus was over against one side of the cell, and the nucleus of the next cell was migrating over a cytoplasmic bridge into the cell in question (fig. 12).

It is likely that this species hybridizes with *B. lenta*. GRAY's manual states: "Trees with characteristics somewhat intermediate between this and *B. lenta* have been called *B. alleghaniensis*." The cytological findings suggest that *B. lutea* may have a heterozygous complex itself. In fact, many prominent evolutionists now consider polyploidy to be directly due to hybridization.

B. grossa Sieb. et Zucca., X-42 (meiosis normal).—Fig. 32 is the heterotypic metaphase plate with forty-two chromosomes. Peculiarly enough, these two hexaploid species exhibit metaphase plates which are easier to count than some of the diploid species, because the spindles are so much broader and the chromosomes not so close together. Cytomyxis and chromatolysis are marked during the prophase, mother cells often appearing as if the entire nucleus had been lost.

DYSPLOID SPECIES

SUBSECTION ALBAE×NANAE (PENTAPLOID×TETRAPLOID)

×*B. sandbergi* Britton. (*B. papyrifera*×*pumila* var. *glandulifera*).—The cytological study of this hybrid has proved to be a distinct correlation and conclusion to the taxonomic studies of ROSENDAHL (39). The latter's observations extended over several years. His drawings of the leaves, catkins, cone scales, and seeds show *B. sandbergi* to possess characters which are intermediate, in every way, between those of the dwarf birch and the paper white birch. ROSENDAHL further points out that there are but three species of *Betula* in Minnesota, where he found this hybrid. These are *B. papyrifera*, *B. lutea*, and *B. pumila* var. *glandulifera*. He has also found and illustrated plants which are undoubtedly hybrids between *B. lutea* and *B. pumila* var. *glandulifera*, and presents photomicrographs of pollen of the three species, each of which shows 100 per cent perfect pollen, and of the two hybrids, which have 33 and 45 per cent of the pollen defective.

The following quotation is from a letter recently received from Professor ROSENDAHL:

> I have just completed another paper, which I mailed last week to Dr. ROBINSON, on experimental evidence of the hybrid nature of *B. sandbergi*. Briefly it relates the results of the cultivation of several F_2 plants of the hybrid showing very clear segregation of the characters of both *B. papyrifera* and *B. pumila* var.

glandulifera. Some of the F_2 plants were grown at the Arnold Arboretum from seed which I sent to Dr. SARGENT. Several years later he sent me specimens grown from these seeds and they show very distinct segregation. I have also found that true *B. pumila* does not occur west of New York state and that specimens from Michigan, Wisconsin, Minnesota, and Indiana which simulate *B. pumila* are nothing but F_2 segregates of *B. sandbergi*. I examined last fall the staminate catkins from one of the F_2 plants growing in our garden and found that all of the pollen grains were irregular in shape and probably all entirely defective. Furthermore, all the seeds from the pistillate catkins examined were found to contain no embryos. If you obtained your material from the Arnold Arboretum [which I did] I am wondering if the plants are not from seed I sent to Dr. SARGENT. In that case you have F_2 plants (assuming that my original seed collection was from an F_1 plant).

Cytology of meiosis in B. sandbergi, 2X-63; X-31, 32 (meiosis very abnormal).—A metaphase plate of a tapetal cell showed sixty-three chromosomes. Diakinesis contains so many elements that it is a most difficult stage to study. The *papyrifera* parent of this hybrid has the haploid number of chromosomes (thirty-five). *B. pumila* has twenty-eight. The variety *glandulifera* has not been available yet, but the chromosome counts of *B. sandbergi* indicate that it must be the same as the regular species.

Apparently all of the chromosomes from the *pumila* parent find synaptic mates and appear during the first division as bivalent chromosomes. The remaining seven chromosomes from the *papyrifera* parent either persist as univalents and lag on the spindle; or two, four, or six of them pair among themselves, forming bivalents. When the latter takes place, thirty-one gemini are formed and the heterotypic division is quite normal, there being only one univalent to lag on the spindle. Fig. 48 shows the chromosome complement of one pole of an early anaphase of the first division, with thirty-one chromosomes; fig. 49 shows the other pole with thirty-two chromosomes. There is always at least one univalent, and, unless it splits, the heterotypic division always produces nuclei with different numbers of chromosomes.

More often the seven extra chromosomes, and sometimes many of the others from *B. papyrifera*, do not pair but remain single and lag on the spindle of the first division (figs. 45, 46). Some bivalents are also seen to be tardy. This tardiness of the chromosomes in their movements on the spindle results in the semiheterotypic division

described under *B. japonica* var. *mandshurica*. The nuclear membrane is formed around the whole spindle, making a single large nucleus, as in fig. 47. This nucleus becomes spherical and passes through the second division as one very broad spindle. Figs. 50 and 51 show the metaphase and anaphase of this restitution nucleus. Some chromosomes lag, possibly because they are univalents which split in the first division and will not split again. The product of this semiheterotypic division is a diad of diploid pollen grains (fig. 52). Between 5 and 10 per cent of the mother cells behave in the manner described.

Occasionally the same sort of thing happens in the homeotypic division. Chromosomes lag, as in fig. 53, and the whole complement of each spindle is included in each of two nuclei. This again results in the formation of a diad of diploid pollen grains. Sometimes only one spindle forms this restitution nucleus, the other forming two nuclei as usual. This results in a triad of pollen grains, one diploid and two haploid in chromosome complement (fig. 56).

More abnormalities occur. During homeotypic metaphase the two equatorial plates frequently unite and form one broad spindle as indicated. At anaphase they are still united, and at telophase the nuclear membrane is laid down around the double chromosome masses again, forming two diploid nuclei and subsequently two large diploid pollen grains. Sometimes two of the spindle poles of the homeotypic anaphase fuse (fig. 55), the other two remaining free and forming haploid nuclei (fig. 56). These several methods of forming diads and triads must produce pollen grains with very diverse genotypic complexes. Another peculiarity was often noted during the homeotypic division. A spindle "fiber" stretched between two poles of different spindles and held several chromosomes, as fig. 57 shows.

It is important to note that these irregularities occur only in the cells which undergo the reduction division. The tapetal cells which divide by mitosis are normal in every way (fig. 58). Apparently these abnormalities are due to non-formation of gemini. ROSENDAHL (39) finds the pollen 45 per cent defective. Chromatolysis is frequently seen during prophase.

B. davurica Pall., X-about 45 (meiosis very abnormal).—Material of this plant has been most difficult to fix and stain properly. This is often the case with plants of heterozygous origin. The first division is characterized by many lagging bivalent and univalent chromosomes (figs. 19, 20). This in itself is strong evidence that the plant is of hybrid origin. Fig. 22 is a heterotypic metaphase plate showing forty-five chromosomes. Staining has been so difficult that it has been possible to count but few plates. The one illustrated is very clear and distinct. Others showed from forty-two to forty-nine chromosomes, but they were not always clear. Subsequent investigation will decide the true number.

Broad spindles with double the number of chromosomes appear frequently, and give rise to diads of diploid pollen grains, just as in *B. japonica* var. *mandshurica* and *B. sandbergi*, both of which are hybrids. An attempt to count one of these broad plates showed about ninety chromosomes.

Cytomyxis and chromatolysis are more marked in this species than in any other studied thus far. At prophase it reaches such proportions that whole anthers of P.M.C.'s are seen to be degenerating, all cells being connected by broad cytoplasmic strands. Fig. 21 shows chromosome migration across a cytoplasmic bridge during the first division. The spindle is involved and the cell to the left seems to be losing its entire chromosome complement to the cell on the right. Likewise, during the first division, the pole of a spindle of a cell in anaphase was seen to be connected by a string of chromosomes to the plate of a cell in metaphase. This may be considered a possible method of an increase in chromosome number. Chains of P.M.C's occur end to end without any callose sheath separating them.

Soon after the tetrads are formed degeneration sets in. It is very unusual to see a tetrad which appears to be normal. Many microcytes and giant cells occur along with the more normal-sized grains. Fig. 17 shows a giant pollen grain, the product of a whole mother cell; fig. 18 indicates the appearance of most grains. They are highly vacuolate and soon degenerate. These conditions present adequate evidence for the consideration of this plant as a hybrid.

Discussion

Since the beginning of the present century some very definite ideas concerning the formation and evolution of species have been materializing. As early as 1906, MONTGOMERY pointed out that chromosomal relations bid fair to become the basis of taxonomy. By 1910 there were many ideas current concerning the origin of species by hybridization. OSTENFELD (32) reviews these and concludes: "New species certainly arise through hybridization, but this method of the origin of species is limited to certain cases, e.g. *Hieracium*, and is checked in many ways." More recently, the same writer (35) said that species descriptions should be accompanied by cytological investigation and that hybridization probably formed new species in the polymorphic genera.

OSAWA (31), examining species of *Taraxacum*, found much abnormality of meiosis in the parthenogenetic *T. albidum*. He notes that amitotic division of some of the daughter nuclei, lagging chromosomes, dwarf nuclei, and polyspory are common to many hybrids; and further holds that species with large chromosome numbers have been derived phylogenetically from those species containing less numerous chromosomes.

WINGE (48) states:

> It must be presumed that occasional hybridization can give rise to the formation of apogamous, or at any rate sexually abnormal "new species," and that these newly formed biotypes and their offspring persist as special minor species or biotypes simply because they are, from their organization, excluded from fertilization, and thus from the adjusting- or mutually supplementing-effect which is, in the writer's opinion, produced by the alternation of generations.

ERNST (11) has written a sizable volume concerning the definite relation between apogamy and hybridity. Apogamous plants are held not to be able to reproduce in the normal manner because they are species hybrids. The two sorts of plants exhibit a cytology which is much alike.

In 1926, DE MOL (30) reported that the first triploid descendants of Dutch flowering bulbs were obtained by union of haploid egg with diploid pollen grain, the latter being produced apparently as a response to physiological conditions induced by artificial shortening of the growing season. He thinks that abnormal physiological states

may play a larger rôle than hitherto supposed in causing chromosome doubling. BLACKBURN and HARRISON (5) explain the formation of a hexaploid rose from crossing a diploid and a tetraploid species. CLAUSEN and GOODSPEED (8), studying *Nicotiana*, found a *glutinosa-tabacum* hybrid (diploid × tetraploid) which doubled all its chromosomes, showed normal divisions, and produced all perfect pollen. This is a similar case to the well-known *Primula kewensis*, which is a tetraploid hybrid resulting from the doubling of all the chromosomes from the diploid and tetraploid parents (DIGBY 10).

GATES (15) says that the time should come when the description of a species is not complete until the morphology of its chromosome group is known. LONGLEY (27), working with maize, finds a reversal of the general idea, in that the more primitive and less specialized members of maize and its relatives have more chromosomes than the more recent and highly specialized species. The same writer later (29) pointed out that dioeciousness is associated with high chromosome number in *Fragaria*. ROSENBERG (38) stated that there is a cytological basis for a new species if the combining gametes have different numbers of chromosomes and the reduction division splits the whole group in half, as in *Papaver*. SHARP (43) gives an interesting review and discussion of the whole question.

TSCHERMAK and BLEIER (47) studied a hybrid between *Aegilops ovata* (diploid) and *Triticum dicoccoidea* (diploid). In F_5 and F_6 generations there was a doubling of chromosomes and the hybrid is a fertile, constant, and intermediate form which breeds true. GOODSPEED and CLAUSEN (18), experimenting with *sylvestris tabacum* crosses in *Nicotiana*, backcrossed with the *sylvestris* parent and found that three general types were produced: (1) abnormal, almost completely sterile forms; (2) individuals more or less closely approximating the F_1 type; and (3) plants more or less identical with *sylvestris*, showing in some cases a considerable degree of fertility, and self fertilization through a number of generations leads to the establishment of fully fertile races identical with *sylvestris*. The *sylvestris* parent has twelve chromosomes after reduction and the *tabacum* parent twenty-four. Seventeen plants produced by backcrossing with *sylvestris* were examined cytologically. They always showed twelve bivalents and a varying number of univalents, from none to

all twelve, and this series varied all the way from *sylvestris* type to aberrant forms. Such work as this is laying a solid foundation for a more perfect understanding of cytological, genetical, and evolutionary phenomena.

KARPECHENKO (**23**), reporting results of crossing *Raphanus* with *Brassica*, states:

> The tetraploid F_2 hybrids do not show segregation, ... acquire quite regular reduction division, full fertility, and, moreover, prove unable to cross with one of their parents, *Brassica*. And it seems that we here approach nearer than we ever did the experimental reproduction of one of the processes in species-formation.

So it is quite clear that the recent work is of such a nature that it cannot contain the slightest provocation for questioning its authenticity.

POLYPLOIDY

Some biologists hold the opinion, with GATES (**15**), that, "In all cases of true polyploidy the increase in chromosome number is by a longitudinal fission of the chromosomes." In many cases, such as in species of *Betula*, it is wholly impossible to ascertain whether or not this condition obtains, on account of the constant size and minuteness of the chromosomes. Also, evidence as to the origin of polyploidy is becoming plentiful, and it does not rely upon the longitudinal fission of chromosomes. I refer to the semiheterotypic division. It is also possible that cytomyxis and chromatolysis may initiate the formation of diploid gametes. Some investigators now believe the underlying principle of polyploidy to rest in hybridization. It is unreasonable then to so restrict, as in the quotation by GATES, the application of a word of such descriptive qualities as polyploidy. Within the last decade or so many outstanding cases of polyploidy have appeared; in fact, it is now seen to be of such widespread occurrence that any genus of a large number of species is justly suspected of containing polyploid members.

GATES (**14**) studied the behavior of the chromosomes in *Oenothera*. *O. gigas* is a tetraploid form of *O. lamarkiana*, being produced by a duplication of the fourteen chromosomes. This *gigas* form, when crossed with the diploid species *O. lata*, produced a triploid which had twenty-one somatic chromosomes, seven of them from *O. lata* and fourteen from *O. gigas*. The germ cells have ten or eleven

chromosomes, which accounts for the fact that different individuals in a race have different chromosome numbers. At once there is light shed on the origin of polyploidy and dysploidy (numerical changes not involving entire sets).

TAHARA (**45**) reported from his investigations on *Chrysanthemum* diploid, triploid, tetraploid, and pentaploid species. In the same year TISCHLER (**46**) produced a remarkable compilation, listing all the chromosome numbers in the plant kingdom which had been determined at the time. No very intensive study is required for one to realize that reduplication of chromosome sets is intimately connected with the cause for the existence of polymorphic groups.

ROSENBERG (**36, 37**) found the reduced number in *Crepis* to be 3, 4, 5, 8, 9, 12, 20, 21, and in *Hieracium* 9, 18, and 21. Species of *Triticum*, with the fundamental number of seven, occur as haploid, diploid, triploid, tetraploid, and hexaploid (SAKAMURA **40**, SAX **42**). LJUNGDAHL (**24**) found the same sort of double series in *Papaver*. The counts during the heterotypic division showed 7, 14, 21, 35, 11, and 22. LONGLEY (**25, 26, 28, 29**) has discovered the following haploid numbers, in *Rubus* 7, 14, 21, 28, 35, 42, 56; in *Zea* 10, 20; in *Citrus* 9, 18; in *Fragaria* 14, 21, 28.

TÄCKHOLM'S work on the Caninae section of *Rosa* (**44**) shows diploid, triploid, tetraploid, pentaploid, hexaploid, and octoploid species and hybrids between them in many different ways. AFZELIUS (1) derived some very interesting haploid counts from species of *Senecio:* 5, 10, 20, 25, 30, 90, also 18, 19, 24, 25, 29. The forms with the latter counts are undoubtedly derived from gametes formed by irregular divisions. CLAUSEN (**7**), in his recent study of *Viola*, reports slight deviations from the cardinal numbers to the following extent: 6, 7, 10, 11, 12, 13, 17, 18, 20, 24, 26, 27, 30, 36.

ORIGIN OF POLYPLOIDY

Non-reduction.—BELLING and BLAKESLEE (**3, 4**) report the absence of the heterotypic division in all four types of *Datura:* haploid, diploid, triploid, and tetraploid. They show that the occurrence of the phenomenon can be intensified by cold. The homeotypic division results in the production of two diploid nuclei which form a diad of large pollen grains.

CHIPMAN and GOODSPEED (6) discuss the cytological features of a *Nicotiana tabacum* var. *purpurea* haploid with twenty-four univalent chromosomes. Often none of them forms bivalents, and the splitting of all the univalents at the first division produces diads of pollen containing the full haploid set of chromosomes.

In ×*Betula jackii*, as noted, there are some P.M.C.'s in which the non-affinity of the chromosomes is accentuated to the extent that no pairing takes place; consequently a broader spindle is formed. All the chromosomes split in this first division, forming a diad of diploid pollen grains.

In *Betula papyrifera* var. *cordifolia*, P.M.C.'s frequently are seen to hold broad metaphase and anaphase plates which contain double the number of chromosomes. Diads of diploid pollen grains are formed. Whether this is to be considered a case of non-reduction is not yet certain. There is evidence that one mother cell may receive the chromosome complement of another by cytomyxis. The diads may also be the consequence of semiheterotypic division, although I have been unable to find any restitution nuclei.

Betula davurica, as noted under the cytology of species of *Betula*, shows exactly the same conditions as *B. papyrifera* var. *cordifolia*. It also produces giant pollen grains, which are tetraploid rather than haploid, being the product of whole P.M.C.'s.

Semiheterotypic division.—ROSENBERG (38) describes this phenomenon. The heterotypic division is prematurely interrupted:

> Round the entire spindle figure a new nuclear wall is formed, resulting in the production of a single large nucleus, the chromosomes of which divide quite in the same manner as in normal interkinesis, but with the diploid number of chromosomes. Such P.M.C.'s divide only once and become dyads thus producing pollen cells with the diploid number of chromosomes. . . . . The cells of the dyads, with the diploid number of chromosomes, very often develop into mature pollen with normal exine.

ROSENBERG worked this out in *Hieracium*.

KARPECHENKO (23) has executed some interesting experiments in crossing *Raphanus* and *Brassica*, and concludes, with reason that he has "experimentally reproduced one of the processes in species formation." The F_1 hybrids exhibit P.M.C.'s which, in some cases, have no union of the nine chromosomes from each parent. The

univalents lag on the spindle, and, except on occasional extrusion, are all included in one nucleus rather than two nuclei. All the chromosomes now divide on one large spindle, forming a diad each cell of which has the somatic number of chromosomes, eighteen. Occasionally irregularities of distribution cause the two pollen grains of the diad to contain seventeen and nineteen, or sixteen and twenty chromosomes.

KARPECHENKO further finds that in the archesporial cells a nuclear division sometimes takes place without cell division. The chromosomes in neither nucleus conjugate, and at the first division the two spindles fuse into one large one. The chromosomes lag so that all are included in one nucleus. At the second division the univalents all split, giving thirty-six chromosomes in each cell of a diad. These pollen grains are tetraploid. A few univalents split at both divisions, making a few extra, and a few are at times extruded so that the number is thirty-six, more or less. KARPECHENKO also finds triploid gametes to be produced. He crossed the *Raphanus-Brassica* hybrid back with *Raphanus*, producing a triploid. The P.M.C.'s showed abnormalities such as lagging and chromosome extrusion. Often the heterotypic division was of the type under discussion and resulted in the formation of a restitution nucleus. The homeotypic division of this nucleus produced a diad of triploid gametes. Although KARPECHENKO finds the triploid and tetraploid forms to occur most frequently in his cultures, there also appear plants with the following constitution: 27–28, 29, 36-38, 38, 40–42, 45, 51–53, 51.

This study is one which provides proof for one of the theories of the origin of polyploidy, namely, hybridization. Its value is accentuated in that it also shows the method involved. The semiheterotypic division is found to produce diploid pollen in two species, the hybrid *B. sandbergi* and the apparent hybrid *B. japonica* var. *mandshurica*. In *B. sandbergi* a restitution nucleus is formed, not only about the first spindle, but also about the homeotypic spindles in cells which complete the heterotypic division, which again results in the production of a diad. In this *B. lenta-pumila* cross the diads make up some 5–10 per cent of the pollen. In *B. japonica* var. *mandshurica* diads often appear in number equal to tetrads.

Other investigators have noted the formation of diploid pollen grains by this semiheterotypic division. BEER (2) worked with *Fuchsia;* YASUI (50) artificially raised *Papaver* hybrids; LJUNGDAHL (24) studied *Papaver;* CHIPMAN and GOODSPEED (6) investigated the *Purpurea* haploid of *Nicotiana tabacum.*

Diploid gametes by fusion of homeotypic spindles.—This is described under *B. sandbergi.* It has also been noted by AFZELIUS (1) in *Senecio,* KARPECHENKO (23) in *Raphanus*×*Brassica,* and by GOODSPEED and CLAUSEN (18) in *Nicotiana.*

Cytomyxis and chromatolysis.—Cytomyxis is protoplasmic continuity, usually in the form of strands, between P.M.C.'s. Apparently it is a widespread condition, which of itself is of no special import. It may be enlarged or aggregated plasmodesmae. Chromatolysis is the dissolution of the karyoplasm. When these two conditions obtain at the same time, and elements from nuclear breakdown migrate across protoplasmic bridges into adjoining cells, the correlation becomes of importance. During the prophases the nucleus is frequently seen to send out long chromatic prolongations which extend across the protoplasmic connections into neighboring mother cells; and occasionally right across the second cell into a third, where it usually ends in a terminal enlargement (fig. 26). I have noted such states in practically all of the species of *Betula* investigated. This has often been reported, the general opinion being to consider such nuclear migrations as abnormalities incited by the action of the fixing fluids.

When a part or the whole of a chromosome set migrates across cytomyctic strands into adjacent cells during the active phases of meiosis, as shown in figs. 11, 12, and 21, it is tempting to look upon such actions as possible cause for the production of other than haploid gametes. In *B. schmidtii* polycary is caused not only by nuclear extrusions, but also by the migration of whole nuclei from cells in the homeotypic telophase across protoplasmic bridges into neighboring cells. Much more observation on these phenomena is necessary before their full significance can be realized.

Giant pollen grains.—The giant grains considered under *B. davurica,* if they affected fertilization, might produce various cases of polyploidy.

Dysploidy and its origin

Dysploidy has often quite apparently arisen from the polyploid species; from one of the gametes having one or two chromosomes (more or less) than the haploid number, due to unequal distribution. In the case of *B. sandbergi*, the combination of tetraploid and pentaploid species could result only in a dysploid species.

Karyoplasmic ratio

Many investigators have noted that a definite relation appears to exist between the number of chromosomes, the size of the nucleus, and the size of the mother cell. Figs. 1–8 clearly illustrate this condition in four of our common northeast American species of *Betula*.

Summary

1. Polymorphic groups of plants are consistently proving to contain species which hybridize readily.

2. A wealth of evidence indicates hybridism to be the cause and polymorphism to be the effect.

3. *Betula* is a highly polymorphic genus.

4. Polymorphic groups usually show polyploidy.

5. *Betula* is a polyploid genus containing diploid, triploid, tetraploid, pentaploid, hexaploid, and dysploid species and hybrids.

6. Species of *Betula* are known to hybridize very readily.

7. The correlation of taxonomic and cytological investigations of ×*B. jackii* and ×*B. sandbergi* presents complete documents of natural hybridization. All of the abnormalities of meiosis which have been considered as characteristic of hybrids have been found to occur in these two species crosses.

8. Hybridization leads to the production of polyploid gametes by the very interesting semiheterotypic division and by non-reduction; consequently heterozygosis is to be considered one of the methods of the origin of polyploidy.

9. Plants, commonly recognized as typical of the following species, are found to exhibit the abnormalities of meiosis which are characteristic of known hybrids and are therefore held to have arisen by heterozygosis: *B. japonica* var. *mandshurica*, *B. davurica*, *B. pendula*, and *B. schmidtii*.

10. Polymorphism in *Betula* is apparently due to the readiness with which the species cross in nature.

11. It follows that *Betula* is another genus in which the multiplication of species has come about, partly at least, by hybridization.

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EXPLANATIONS OF PLATES XI, XII

Phases of microsporogenesis in *Betula* species; ×2200 unless otherwise stated. Note proportional increase in cell size and breadth of equatorial plate as the number of chromosomes increases from 14 to 28 to 35 to 42 in four of our common northeastern species of *Betula* (figs. 1–8).

PLATE XI

Betula populifolia Marsh.

FIG. 1.—P.M.C. at heterotypic metaphase.

FIG. 2.—Same from polar view showing 14 chromosomes.

Betula pumila L.

FIG. 3.—P.M.C. at heterotypic metaphase.

FIG. 4.—Same from polar view showing 28 chromosomes.

Betula papyrifera Marsh.

FIG. 5.—P.M.C. at heterotypic metaphase.

FIG. 6.—Same from polar view showing 35 chromosomes.

Betula lutea Michx. f.

FIG. 7.—P.M.C. at heterotypic metaphase.

FIG. 8. Same from polar view showing 42 chromosomes.

Betula papyrifera var. *cordifolia* (Regel) Fernald

FIG. 9.—Heterotypic metaphase plate showing 28 chromosomes.

FIG. 10.—Metaphase plate of cell which did not complete reduction division, showing 56 chromosomes.

FIG. 11.—Three cells undergoing chromatolysis during homeotypic division, showing migration of chromosomes; ×1600.

Betula lutea Michx. f.

FIG. 12.—Cytomyxis and chromosome migration, one cell losing its chromosome complement to its neighbor; ×1600.

Betula japonica Sieb.

FIG. 13.—Heterotypic metaphase plate showing 14 large chromosomes.

Betula japonica var. *mandshurica* (Regel) H. Winkl.

FIG. 14.—Heterotypic metaphase plate showing 42 chromosomes, 14 of them large.

FIG. 15.—Heterotypic metaphase showing lagging univalents; ×1600.

Fig. 16. Heterotypic anaphase showing lagging chromosomes; ×1600.

Betula davurica Pall.

FIG. 17.—Giant pollen grain, product of whole mother cell; ×500.

FIG. 18.—Pollen grain normal in size, highly vacuolate; ×500.

FIG. 19.—Heterotypic metaphase showing lagging bivalents and univalents; ×1600.

FIG. 20.—Heterotypic anaphase showing lagging bivalents and univalents; ×1600.

FIG. 21.—Chromosome migration between two cells during heterotypic metaphase; ×1600.

FIG. 22.—Heterotypic metaphase plate showing 45 chromosomes.

Betula schmidtii Regel

FIG. 23.—Phase corresponding to heterotypic metaphase showing bivalents and univalents lagging all over spindle.

FIG. 24.—Polycaric mother cell resulting from chromosome extrusion.

FIG. 25.—Heterotypic metaphase plate of normal division showing 14 chromosomes.

Betula pendula Roth.

FIG. 26.—Chromatolysis in mother cells previous to heterotypic division; ×1100.

Betula nigra L.

FIG. 27.—Heterotypic metaphase plate showing 14 chromosomes.

Betula coerulea Blanchard

FIG. 28.—Heterotypic metaphase plate showing 14 chromosomes.

Betula coerulea-grandis Blanchard

FIG. 29.—Heterotypic metaphase plate showing 14 chromosomes.

Betula pendula Roth.

FIG. 30.—Diakinesis showing 14 pairs of chromosomes.

Betula maximowicziana Regel

FIG. 31.—Heterotypic metaphase showing 14 chromosomes.

Betula grossa Sieb. et Zucc.

FIG. 32.—Heterotypic metaphase plate showing 42 chromosomes.

PLATE XII

Betula jackii Schneid. (*B. lenta*×*pumila*)

FIG. 33.—Diakinesis showing univalent, bivalent, trivalent, and quadrivalent chromosomes.

FIG. 34.—Phase corresponding to heterotypic metaphase, chromosomes scattered all along spindle (note bivalents and univalents).

FIG. 35.—Heterotypic metaphase plate showing 42 chromosomes, about two-thirds of them large, the others smaller.

FIG. 36.—Heterotypic anaphase showing lagging univalents and undivided bivalents (non-disjunction).

FIG. 37.—Heterotypic metaphase plate showing 28 chromosomes.

FIG. 38.—Metaphase of second division of cell with broad plate containing unreduced number of chromosomes.

FIG. 39.—Homeotypic anaphase showing lagging chromosomes.

FIG. 40.—Polyspory: 6 vari-sized pollen grains arising from one mother cell.

Betula pumila L., maternal parent

FIG. 41.—Heterotypic anaphase (note regularity).

FIG. 42.—Heterotypic metaphase plate containing 28 large chromosomes.

Betula lenta L., paternal parent

FIG. 43.—Heterotypic metaphase plate showing 14 small chromosomes.

FIG. 44.—Heterotypic metaphase (note regularity).

Betula sandbergi Britton (*B. papyrifera*×*pumila* var. *glandulifera*); ×1600

FIG. 45.—Heterotypic metaphase showing lagging bivalent and univalent chromosomes.

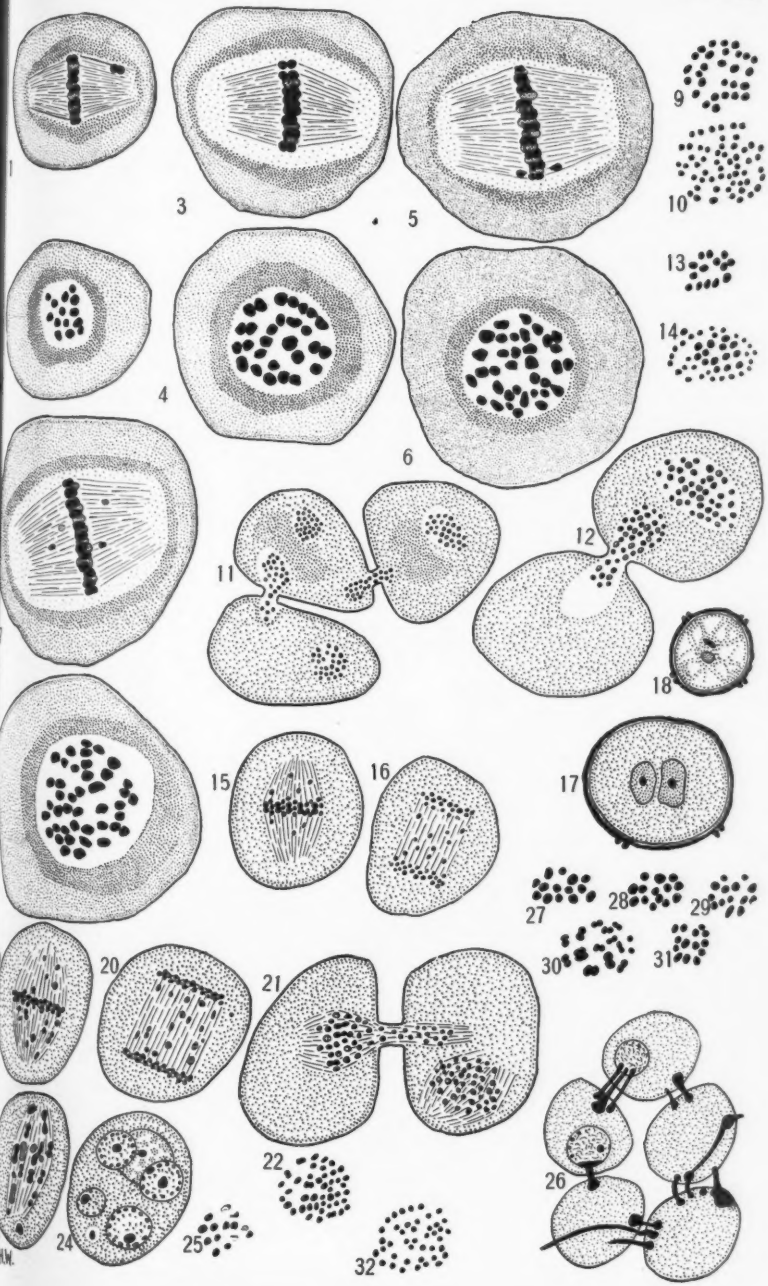
FIG. 46.—Heterotypic anaphase showing lagging bivalent and univalent chromosomes.

FIG. 47.—Restitution nucleus near close of semiheterotypic division.

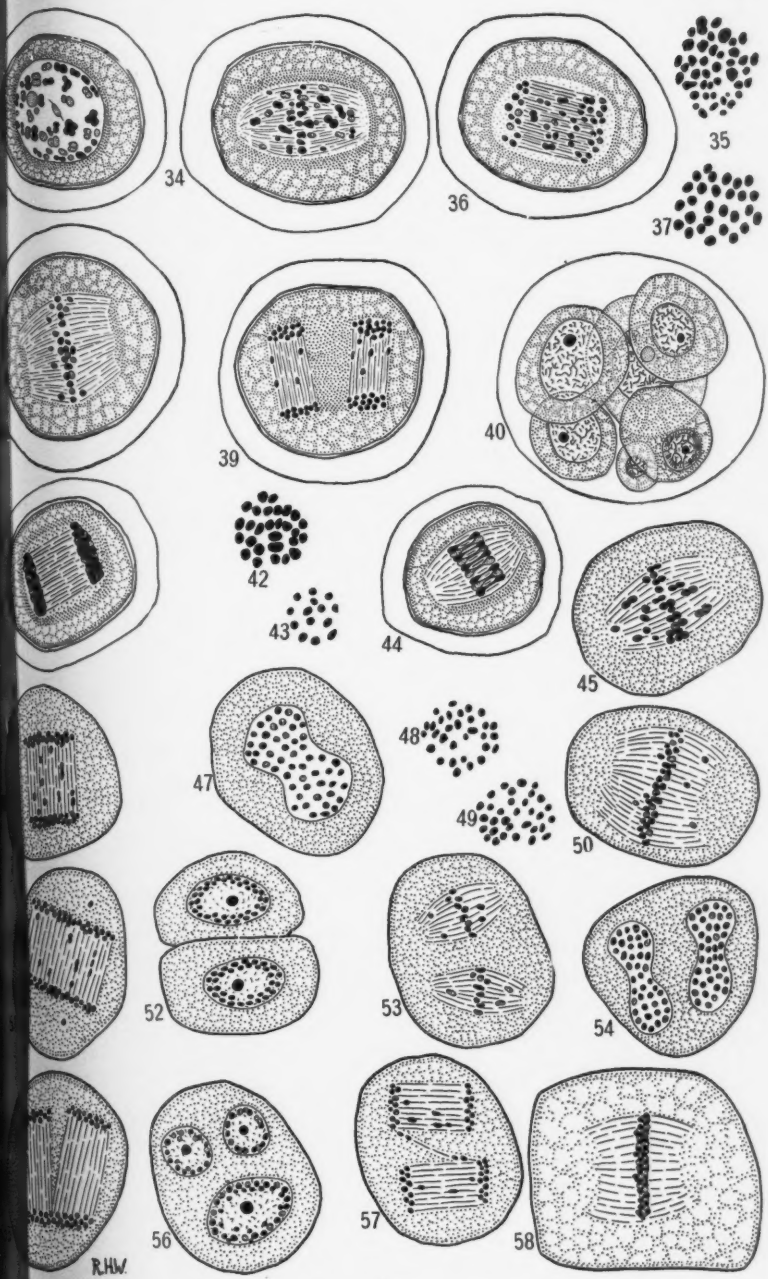
FIG. 48.—Heterotypic early anaphase plate with 31 chromosomes.

FIG. 49.—Same with 32 chromosomes.

FIG. 50.—Metaphase of second division in cell containing unreduced nuclear complement, chromosomes which would normally occupy two spindles are all on one very large spindle.



WOODWORTH on BETULACEAE



WOODWORTH on BETULACEAE

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FIG. 51.—Anaphase of broad plate containing unreduced nuclear complement (note many lagging chromosomes).

FIG. 52.—Diad of diploid pollen grains; product of semiheterotypic division.

FIG. 53.—Homeotypic metaphase of cell not undergoing semiheterotypic division; chromosomes lagging.

FIG. 54.—Two restitution nuclei of a semihomeotypic division.

Fig. 55.—Homeotypic anaphase showing nuclear complement of two poles fused.

FIG. 56.—Result of fusion showing one diploid nucleus and two haploid nuclei.

FIG. 57.—Homeotypic anaphase showing chromosomes lagging on spindle and peculiar connection of the two spindles by a "fiber" which holds four chromosomes.

FIG. 58.—Metaphase of tapetal (sporophytic) cell (note characteristic normality of division).

COMPOSITION OF WALNUT TREES AS AFFECTED BY CERTAIN SALTS[1]

A. R. C. Haas

(WITH THREE FIGURES)

Introduction

It is a matter of common observation that walnut trees are extremely sensitive to accumulations of certain salts in the soil. The accumulation in soils of unfavorable salts, such as chlorides or sulphates, may result from frequent irrigation with saline water, faulty fertilizer practice, as well as poor drainage, etc. Under such conditions the cycles of growth may be so short as to give the tree a stunted appearance. In extreme cases the trunks fail to increase appreciably in diameter, and together with the branches they may be covered with a dense growth of lichens.

The leaves of walnut trees growing in soil containing considerable saline material may be undersized and may have a wilted appearance. The leaves are most frequently burned along the tip and margins and in extreme cases are also burned between the veins. Premature abscission of the leaves may occur, and this often leads to the production of late growth which matures poorly and is very susceptible to injury by freezing.

The tree materials studied in the present investigation were obtained from walnut trees grown in controlled soil and sand cultures, and also from trees in affected walnut groves. Comparisons have been made in some cases with results obtained from various species of fruit trees which had grown under similar conditions of exposure to saline soil.[2] More questions have been raised than have been answered and further studies are to be desired.

Very little has thus far been done in gaining an understanding of

[1] Paper no. 193, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] Through the kindness of Mr. Ralph LaRue, then of the field department of the California Walnut Growers Association, the writer has been brought into contact with the effects of saline soil conditions upon walnut trees in the field.

the effects of salts on trees, and the present preliminary study upon walnut trees is concerned with only one phase, that of chemical composition and behavior of the trees.

Method for large tank cultures

At the outset of this investigation, controlled experiments were carried on with walnut trees in soil in large galvanized iron tanks. The containers were cylindrical in shape and were 44 inches in diameter and 52 inches deep. The bottom of each tank tapered toward the center, where a heavy perforated brass plate covered the opening leading into a 2-inch drainage pipe. The surfaces of the tanks were heavily coated with asphalt paint, and when dry the tanks were placed in the soil to within 6 inches of the top of the tanks. Some finely crushed granite was placed in the bottom of each to promote drainage, and then soil was placed in each tank and firmly tamped as the tanks were being filled. A Placentia walnut tree budded on black walnut stock was planted in each tank in March, 1920. Poultry netting, 2 feet high and $\frac{3}{4}$-inch mesh, with the upper edge bent down and outward, was placed about the upper rim of each tank as a protection against rodents, etc., and the treatment was begun.

In the first experiments, large quantities of soil were transported from the field and placed in the tanks in the same order, so far as possible, as the soil layers existed in the field. In no case could tree behavior be obtained, by growing walnut trees in these tanks, that was at all similar to that of the trees growing on this same soil in the groves from which the soil was obtained. Also it was found in preliminary studies that soils, obtained from walnut groves where poor tree growth prevailed, were distinctly improved for tree growth upon being transported to large tanks. The biological oxidation, the redistribution and reduction of localized high salt concentrations, and other factors no doubt play a large part in such soil improvement.

It was found that the heavy types of soil were not well adapted to tank culture work on account of the enormous shrinkage from the inner surface of the tanks and the tendency of such soils to produce large cracks as they dried somewhat. This made it extremely difficult to apply solutions to the soil and have the soil retain them

instead of their running directly into the drainage system. It was found that Ramona loam from the Box Springs tract of the Citrus Experiment Station and Sierra loam from the Rubidoux tract were both very satisfactory for tank cultures.

Six tanks were filled with Ramona loam soil taken from near the Box Springs Station tract, and a budded walnut tree was planted in each on March 30, 1920. A water extract of the soil used in filling the tanks gave the following results in terms of parts per million of dry soil: CO_3, 0; HCO_3, 183; NO_3, 0; Cl, 18; SO_4, 22; PO_4, 18; total solids, 400.

TREATMENT OF TANK CULTURES

Three of the tanks (13, 14, and 15) received tap water having a pH of 7.7, while three of them (16, 17, and 18) received a synthetic

TABLE I

COMPOSITION OF IRRIGATION WATER (PARTS PER MILLION)

	Na	Ca	Mg	CO_3	HCO_3	NO_3	Cl	SO_4	Total solids
Irrigation water used in unthrifty walnut grove	221	128	163	0	308	10	240	759	1927
Irrigation water (used in tanks 16–18) made artificially to imitate that used in unthrifty grove	259	153	158	0	538	12	253	756	2108
Tap water*	89	50	18	0	236	....	43	61	408

* Analysis of tap water January 26, 1916, by the Department of Chemistry, Citrus Experiment Station.

irrigation water made by adding salt solutions and tap water together so as to secure a certain ion concentration. This synthetic irrigation water (pH 7.8) was made to imitate the composition of water used to irrigate a soil near Whittier, California, on which walnut trees were most unthrifty.

Table I gives the ion concentration of the synthetic irrigation water given the soil in tanks 16–18. Each of the six tanks received at intervals the same amount of Hoagland's nutrient solution. It was observed that the water on the soil in tanks 16–18 had a greater rate of percolation than that of the control tanks 13–15, due no doubt to the flocculation brought about by the increased concentration of bases in the soil solution.

In July, 1920, the soil of all of the tanks was sampled and analyzed, and in table II are shown results typical of the two series. The use of this irrigation water from April to July brought about considerable increases in the water-soluble salts.

After the initial wetting of the soil in each tank with tap water over a period from March, 1920, to October, 1925, approximately 4500 liters of tap water and 2250 liters of Hoagland's solution made up with the tap water were added to each of tanks 13–15, and about

TABLE II

COMPOSITION OF A ONE-TO-FIVE WATER EXTRACT OF SOIL IN TERMS OF PARTS PER MILLION OF DRY SOIL

	Average of tanks 13–15 (control)				Average of tanks 16–18 receiving saline irrigation water			
	Depth in feet							
	1	2	3	4	1	2	3	4
Na	30	16	19	10	66	20	39	21
Ca	21	23	19	20	62	35	50	27
Mg	9	8	8	6	21	12	18	9
Cl	23	27	21	21	71	41	44	27
HCO_3	82	70	92	73	76	76	76	92
NO_3	24	13	20	16	31	22	12	9
SO_4	29	22	6	1	18	53	123	27
SiO_2	35	30	20	26	28	41	35	33
Total solids as sulphates	208	169	164	128	518	241	378	203
pH	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.6

9400 liters of the synthetic irrigation water and 2250 liters of Hoagland's solution were added to each of tanks 16–18. The plan was to give each of tanks 13–15 tap water and occasionally Hoagland's solution, and when this was done, a like amount of Hoagland's solution was always at once added to each of tanks 16–18, plus additions of synthetic irrigation water.

Drainage water from tanks

The average results of analyses of the drainage water from tanks 13–15 and 16–18 are given in table III. They show the concentration of the various ions in the drainage water, and in particular the increasing concentration of total solids with time when a saline irrigation water is used. Headley (3) has shown that growth is more

closely related to the proportion of salts recoverable from the soils than to the proportion of salts added.

GROWTH UNDER SALINE CONDITIONS

Fig. 1 shows the effect of the saline irrigation water on the total growth during 1921 of the walnut trees in tanks of soil. The curves each represent the average total growth of the shoots of three walnut trees in tanks of soil. Curves *B* and *C* are for trees in Yolo clay loam

TABLE III

COMPOSITION OF DRAINAGE WATER IN TERMS OF PARTS PER MILLION OF SOLUTION

	3/30–4/30/1920		5/1–6/21/1920		6/22–9/25/1920		10/1/1921		10/1925	
	13–15 (CONTROL)	16–18 (SALINE IRRIGATION WATER)	13–15 (CONTROL)	16–18 (SALINE IRRIGATION WATER)	13–15 (CONTROL)	16–18 (SALINE IRRIGATION WATER)	13–15 (CONTROL)	16–18 (SALINE IRRIGATION WATER)	13–15 (CONTROL)	16–18 (SALINE IRRIGATION WATER)
Na	13	58	82	74	57	20	71	199	243	2333
K	.....	.....	.....	.....	.....	.....	.....	.....	41	247
Ca	73	95	122	103	159	389	96	927	77	1374
Mg	47	22	16	21	39	122	32	291	50	652
Cl	22	19	20	23	67	358	66	1274	241	2957
HCO_3	311	356	476	440	482	578	311	336	.....	.....
NO_3	6	98	71	35	152	124	89	216	.....	.....
SO_4	58	54	69	78	64	417	71	1501	344	5252
PO_4	.....	.....	.....	.....	3	1	.....	.....	.....	.....
Total solids as sulphates	666	553	729	631	1034	2808	702	5280	815	13150
pH	8.2	8.2	7.6	8.1	7.0	7.7	7.8	7.8	.....	.....

soil secured at two different locations near Whittier, California, and used in tank cultures; curve *A* represents the control trees (13–15); curve *D* represents the trees (16–18) in soil that received saline irrigation water. In the course of less than two years' use of the saline irrigation water, the soil solution seems to have somewhat reduced the growth rate.

The increase in the concentration of water-soluble salts in the soil receiving irrigation water brought about certain effects upon the walnut trees. According to the quantities of solution applied to the soil, trees 16–18 must have been less economical of the water supplied in relation to the growth produced. By the use of cobalt chloride

paper, a large number of transpiration tests were carried out upon the leaves of trees both in the control series and in that receiving saline irrigation water. Such rough tests indicated that the evapora-

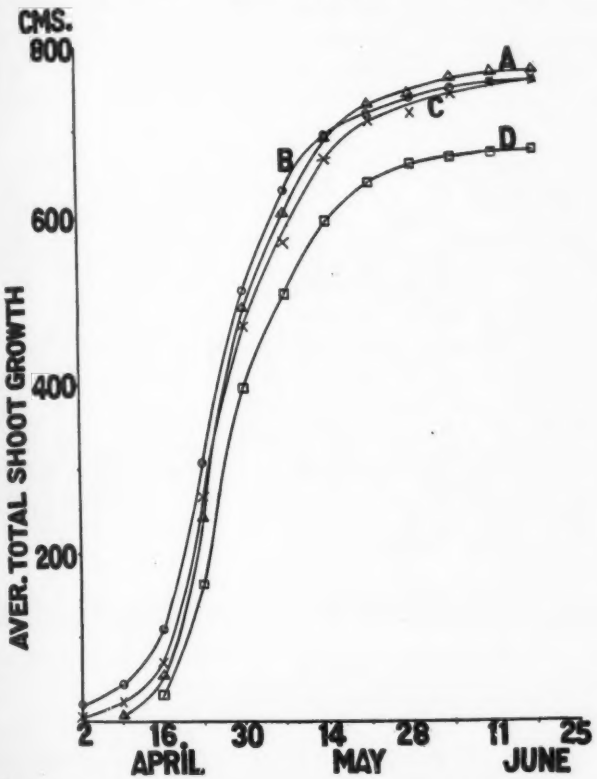

FIG. 1.—Effect of saline irrigation water on average total shoot growth of walnut trees: curves *B* and *C* represent average for trees in two different lots of Yolo clay loam soil obtained near Whittier, California; curves *A* and *D* are for trees in Ramona loam soil from Riverside, California. Hoagland's solution and tap water used for all trees; in addition, trees represented by curve *D* were given saline irrigation water.

tion rate from the leaves of the control trees was slightly less than, or equal to, that from the surface of a moist blotter; but that the evaporation rate from the leaves of the trees in soil receiving saline

irrigation water was somewhat greater than that from a moist blotter.

In October, 1925, the experiment was terminated. The root systems of trees 16–18, in soil irrigated with saline water, were much reduced, weighing an average of 13 pounds each; while those of the control trees 13–15 weighed an average of 20 pounds each. The average circumference 3 inches above the bud union was 20.1 inches for

FIG. 2.—Effect of saline irrigation water on growth of walnut trees: left, tree 15, control in soil receiving Hoagland's solution and tap water; right, tree 16, in soil receiving Hoagland's solution and synthetic saline irrigation water.

trees 13–15 and 15.9 for trees 16–18. The average weight of the tops without the leaves for trees 13–15 was 14 pounds and for trees 16–18 was 6 pounds.

The saline irrigation water had an effect upon the size of walnut leaves, as shown in table IV. It was found that the leaves of trees in soil receiving saline water were smaller on successive cycles of growth. No mottling or "walnut yellows" was present. Slight burning of the leaves of trees 16–18 was seen during the last year of the experiment. Fig. 2 shows the dead shoots and the sparse reduced-

sized foliage of tree 16 as compared with tree 15; also the smaller diameter of the trunk about the bud union is clearly evident.

Distribution of inorganic constituents of walnut shoots at end of growing season

Very little is known in regard to the effect of age on the composition of matured cycles of growth of walnut shoots. To gain such knowledge, composite samples of the cycles of growth for each group

TABLE IV

Effect of saline irrigation water upon size of walnut leaves

Size of average leaflets of 25 whole walnut leaves taken July 29, 1925, from control trees 13–15*

No. measured to obtain size of average leaflet	Arrangement and designation	Designation	Average length (cm.)	Average width (cm.)
23	1	1	12.6	6.1
25		2	12.9	5.7
25	2—3	3	12.9	5.6
25		4	12.9	5.7
25	4—5	5	13.0	5.8
24		6	10.6	4.9
23	6—7	7	10.6	5.0
18		8	7.5	4.1
20	8—9	9	7.9	3.8
1		10	8.0	4.0
1	10—11	11	8.0	4.0

Size of average leaflets of 25 whole walnut leaves taken July 29, 1925, from trees 16–18 that received saline irrigation water†

25	1	1	7.3	2.9
25		2	7.7	3.0
24	2—3	3	7.8	3.3
25		4	7.7	3.3
24	4—5	5	7.6	3.3
24		6	5.4	2.6
24	6—7	7	5.4	2.6
10		8	4.7	2.1
10	8—9	9	4.5	2.3

* Average length of leaf stalk (25 whole leaves) 26.3 cm.
† Average length of leaf stalk (25 whole leaves) 14.4 cm.

of trees were obtained by pruning off the cycles in the order of their occurrence, from the shoot tips toward the base of the trunks. The growth of the shoots of walnut trees 13–15 and 16–18 usually consisted of only one cycle a year. An analysis of the samples is given in table V. It will be seen that, with the exception of the youngest cycles of growth, the ash as a percentage of the dry matter in trees 13–15 decreases from the youngest growth down to the coarse root-

lets. A similar condition exists in the samples from trees 16–18, except that here the decrease takes place in both directions, from the young shoots toward the root and from the coarser rootlets toward the trunk, with a minimum near the bud union or ground level. The roots of trees 16–18 were bathed by a soil solution extremely concentrated as regards certain salts. The gradient in the ash content is not so easily followed when the salts of the ash are studied,

TABLE V

EFFECT OF AGE AND SALINE SOIL ON COMPOSITION OF WALNUT SHOOTS

	November 13, 1925	Ash as percentage dry matter	Percentage dry matter						
			Total Cl	Total S as SO_4	Total P as PO_4	Ca	Mg	Na	K
Trees 13–15, in soil receiving good irrigation water	Apical (last) cycle	7.42	0.07	0.28	1.56	2.13	0.39	0.04	0.59
	Seventh cycle	6.55	.03	.18	0.74	.....	.....	.....	.....
	Sixth cycle	7.07	.04	.19	.....	2.38	.21	.06	.39
	Fifth cycle	6.83	.02	.14	.70	.....	.....	.....	.....
	Fourth cycle	6.41	.02	.17	.70	2.17	.20	.11	.33
	Third cycle	5.97	.02	.13	.81	1.96	.20	.16	.35
	Second cycle	5.90	.01	.12	.82	.....	.....	.....	.....
	First cycle	4.71	.....	.25	.67	1.44	.22	.17	.27
	Main root	4.53	.....	.....	.....	0.92	.17	.13	.72
	Rootlets	4.50	.03	.12	0.64	1.27	.10	.10	.51
Trees 16–18, in soil receiving saline irrigation water	Apical (last) cycle	6.30	.49	.74	1.14	1.20	.80	.17	.54
	Sixth cycle	4.75	.34	.52	0.76	.....	.....	.....	.....
	Fifth cycle	7.76	.....	.39	.98	2.34	.39	.11	.29
	Fourth cycle	6.15	.....	.42	.98	.....	.....	.....	.....
	Third cycle	5.64	0.20	.32	.96	1.72	.31	.15	.28
	Second cycle	5.14	.....	.....	.....	.....	.....	.....	.....
	First cycle	4.96	.....	.....	.....	1.48	.29	.27	.20
	Main root	5.07	.....	.....	.....	0.77	.32	0.35	.64
	Rootlets	6.61	1.19	0.44	0.57	1.22	0.22	1.00	0.38

for some of the constituents of the ash or dry matter are decreasing while others are increasing. The high values of the magnesium, total chlorine, sulphur, and phosphorus in trees 16–18 are outstanding. The gradients observed are indicative of the interdependence of the nutrition of one cycle of the tree on those that have preceded. The importance of this fact is often overlooked in reclamation studies upon salt-affected trees, the recovery of which is looked for at too early a date, long before the external and internal equilibria have had time for sufficient change to take place and reflect itself in improved appearance of the trees.

Inorganic composition of woody shoots in spring, and changes due to growth

The results given in table VI make clearer the effect of growth under normal conditions upon the composition of the tree, and the effect of the addition of a new cycle upon the composition of the

TABLE VI

Effect of growth upon composition of mature prior cycles of walnut shoots from healthy tree

	Mature last cycle of growth of 1925, removed for analysis May 4, 1926, before new growth had begun			Last cycle of growth of 1925, removed for analysis June 15, 1926, after new cycle had been made from terminal bud and had ceased elongating		
	Soluble fraction (per cent)	Insoluble fraction (per cent)	Percentage	Soluble fraction (per cent)	Insoluble fraction (per cent)	Percentage
Ash as percentage of dry matter	1.98	8.88	.....	1.36	6.80	.....
Ash of soluble fraction as percentage of total ash	18.22	.....	.....	16.63	.....	.....
Total Ca as percentage of total ash	.....	.....	33.56	.....	.....	34.37
Percentage of total Ca in each fraction	8.12	91.88	.....	6.28	93.72	.....
Ca of each fraction as percentage of ash of same fraction	14.95	37.70	.....	12.98	38.64	.....
Percentage of total Mg in each fraction	61.36	38.64	.....	42.48	57.52	.....
Total Mg as percentage of total ash	.....	.....	4.05	.....	.....	2.37
Percentage of total Na in each fraction	65.18	34.82	.....	53.70	46.30	.....
Total Na as percentage of total ash	.....	.....	2.28	.....	.....	2.65
Percentage of total K in each fraction	87.39	12.61	.....	91.38	8.62	.....
Total K as percentage of total ash	.....	.....	3.29	.....	.....	5.41
Total P as percentage of dry matter	0.23	0.21	.....	0.13	0.11	.....

cycle from which it took origin. In this table composite samples consisting of twenty-five shoots of a given cycle from a healthy walnut tree on the Rubidoux tract were used. After a new cycle is produced, the ash of both the water-soluble and water-insoluble fractions is reduced. Practically no change takes place in the total calcium as percentage of total ash, or in the general relationship involving calcium. The percentage of total magnesium in the ash of a cycle is reduced after a new cycle has grown from it, and this is largely repre-

sented by the migration of water-soluble magnesium. The total potassium as a percentage of the ash is increased after new growth is produced. A decided loss of total phosphorus takes place in both fractions of the dry matter of a shoot when new growth is produced from it. It is evident that the growth of one portion of the tree brings about changes in the composition of older portions, and brings about changes in the equilibrium conditions within the tree and no doubt also in the soil solution.

Effect of saline irrigation water on composition of walnut leaves from trees in tank cultures

The saline condition of the soil is reflected not alone in the composition of the woody growth cycles that compose the shoots (table V), but also in the marked changes that take place in the composition of the leaves. Samples of leaves of the same age were taken from the trees of both series, 13–15 and 16–18, and analyses were made of their ash (table VII). The ash of the walnut leaves from trees 16–18, when calculated as a percentage of the dry matter, is always lower than that of the control trees 13–15, from which one may infer that the organic matter of the dry leaves from trees 16–18 was greater than that of the leaves from the control trees 13–15. This was at least expected on account of the high concentration of the soil solution in tanks 16–18, and is therefore of unusual interest. The amount of sodium present in the leaves of both series is not very large, when we consider that sodium was the base applied in the largest amount in the irrigation water. The amount of potassium in the dry matter of the leaves of trees from tanks 13–15 is always larger than that of leaves from the trees in tanks 16–18 that were irrigated with saline water free from potassium. Table III shows that in October, 1925, the drainage water from tanks 16–18 contained very much larger concentrations of sodium and potassium than that from tanks 13–15, and yet the leaves from trees 16–18 contained less potassium than those from trees 13–15. This is no doubt due in part to the reduced ash content of the leaves from trees 16–18, and in part to the small amount of potassium present in the soil solution, as indicated by the drainage water in comparison with the large concentration of other bases. It will be observed that near the start of the experiment

the potassium present in the leaves of trees 16–18 approached that present in the leaves of trees 13–15, but that it fell off considerably as the trees became affected by the saline irrigation water.

It is of interest that the calcium content of the dry matter of the leaves of trees 13–15 is always greater than that of trees 16–18. A reduced calcium content is also typical of walnut leaves affected with "yellows" (HAAS, BATCHELOR, and THOMAS 2), the symptoms of

TABLE VII

EFFECT OF SALINE IRRIGATION WATER UPON ASH COMPOSITION OF WALNUT LEAVES

TREES*	DATE OF SAMPLING	ASH AS PERCENTAGE OF DRY MATTER	GRAMS IN 100 GM. OF DRY MATTER (CALCULATED FROM ASH ANALYSES)						
			Na	K	Ca	Mg	Cl	SO_4	PO_4
Control	Nov. 2, 1922	9.29	0.250	1.095	2.035	0.430	0.137	0.206	0.602
Treated	Nov. 8, 1922	8.64	.284	1.063	1.620	.626	.698	.208	.444
Control	July 28, 1923	9.84	.367	1.635	1.932	.458	.110	.272	.678
Treated	July 28, 1923	7.17	.362	1.387	1.001	.622	.484	.284	.529
Control	Nov. 15, 1923	9.77	.285	1.192	2.228	.420	.188	.165	.794
Treated	Nov. 15, 1923	6.87	.078	0.508	1.370	.708	.517	.177	.491
Control	Dec. 3, 1924	10.13	.....	.....	2.247	.492	.366	.270	.649
Control	Nov. 19, 1924	9.49	.313	1.125	1.929	0.410	.259	.241	.742
Treated	Nov. 19, 1924	8.52	.202	0.667	1.320	1.126	.943	.405	0.491
Control	July 29, 1925	8.28	.337	1.295	1.534	0.429	.106	.267	.....
Treated	July 29, 1925	6.96	0.249	0.880	0.916	0.880	0.747	0.338	.....

*Control, trees 13–15, Hoagland's solution with tap water; treated, trees 16–18, same with added salts.

which were absent from these trees. In walnut leaves affected with yellows, besides a reduced calcium content there is an increased potassium content, a condition which was not found in trees 16–18. It seems, therefore, that this is further evidence, although indirect, that walnut leaves affected with yellows are characterized not only by a reduced calcium content but also by an increased potassium content.

The magnesium content of the ash of the leaves of trees 16–18, in soil receiving saline irrigation water, was considerably higher than that of trees 13–15; and in the last two samples of leaves from trees 16–18 the content of magnesium approached that of calcium.

The large concentration of chlorine in the drainage water (table III) was accompanied by increased concentrations of inorganic

chlorine in the leaves of trees 16–18. The concentration of sulphate in the drainage water also was large, and in fact usually exceeded that of chlorine. In the first three samplings of leaves from trees 16–18, however, the increases in the sulphate content of the ash over that of leaves from trees 13–15 were extremely small. The phosphate content of the ash, when calculated as a percentage of the dry matter, is much reduced in the leaves of trees 16–18 below that of leaves from trees 13–15. The organic chlorine, sulphur, and phosphorus in walnut and other tree leaves will be referred to later.

The effect of irrigation water rich in sodium, calcium, magnesium, chlorine, and sulphate upon the ash composition of the leaves of the experimental trees is a reduction in the content of calcium and an increase in the content of chlorine and sulphate. It is of interest that the sodium, potassium, and calcium contents of the affected leaves are actually reduced while that of magnesium is increased. In table VII is seen the great uniformity that exists in the concentration of magnesium in the dry matter of the leaves taken from the control trees 13–15, and the great variation in that of the leaves taken from trees 16–18.

Effect of saline irrigation water on composition of walnut fruits from trees in tank cultures

A comparison of the analyses of the ash of walnut husks, shells, and kernels from both sets of trees is given in table VIII. The ash of the husks as a percentage of the dry matter is least in the case of the trees that grew in soil receiving saline irrigation water, which is not what one would expect to find. The husks of trees 16–18 contain less sodium, potassium, and calcium, but more magnesium, chlorine, and sulphate than those of trees 13–15. The same relationship holds for the composition of the shells as for the husks, except in the case of sulphur. The dry matter of the kernels from nuts of trees 16–18 in 1923 contains less ash, and the ash contains less potassium, calcium, and phosphorus than in the case of trees 13–15. In 1923 the kernels from trees in both series were plump and uniform. In 1925 the kernels from trees 13–15 were plump, however, while those from trees 16–18 were somewhat shriveled and dark brown. The shriveling and discoloration explain the fact that the ash constituents

of the kernels (on the basis of dry matter) from trees 16–18 exceed in every case those of the kernels from trees 13–15; the ash is increased while the dry matter is decreased. A more detailed investigation of the shriveling of walnut kernels has been made by HAAS and BATCHELOR (1).

Attention should be called to the striking situation that exists in walnut husks. The husks are green, and very likely take part in

TABLE VIII

EFFECT OF SALINE IRRIGATION WATER UPON ASH COMPOSITION OF WALNUT HUSKS, SHELLS, AND KERNELS

MATERIAL	TREES*	DATE OF SAMPLING	ASH AS PERCENTAGE OF DRY MATTER	GRAMS IN 100 GM. OF DRY MATTER (CALCULATED FROM ASH ANALYSES)						
				Na	K	Ca	Mg	Cl	SO_4	PO_4
Husks	Control	Oct. 1923	14.60	1.6575	6.4615	0.7340	0.1410	0.0910	0.3250	0.497
	Treated		12.28	1.4345	5.4315	.459	.304	.420	.446	.295
	Control	Sept. 9, 1925	12.08	1.635	6.044	.354	.104	.085	.336	.615
	Treated		9.30	1.151	4.518	.156	.245	.765	.521	.722
Shells	Control	Oct. 1923	1.68	0.130	0.425	.284	.039	.007	.016	.045
	Treated		1.29	.055	.329	.210	.046	.011	.014	.007
	Treated	Nov. 6, 1924	1.25	.083	.364	.145	.051	.021	.0130	0.047
Kernels	Control	Oct. 1923	2.03	.121	.487	.102	.176	.005	.026	1.271
	Control		2.12	.107	.482	.096	.169	.004	.010	1.349
	Treated		1.68	.091	.418	.069	.164	.004	.025	.....
	Treated		1.74	.114	.415	.083	.165	.005	.015	1.084
	Control	Sept. 9, 1925	2.31	.060	.480	.124	.184	.004	.016	1.096
	Treated		3.45	0.140	0.820	0.2370	0.2110	0.0280	0.063	1.159

*Control, trees 13–15, Hoagland's solution with tap water; treated, trees 16–18, same with added salts.

photosynthetic processes to some extent, like leaves. The dry matter of the husks of trees 13–15, however, contains about 1.6 per cent of sodium and about 6 per cent or more of potassium. The calcium concentration in the dry matter of the husks is very low in comparison with that of sodium and potassium. The ash content of the dry matter of the husks usually greatly exceeds that of the dry matter of the leaves. In the dry matter of good walnut leaves (table VII) the concentration of calcium is much greater than that of potassium while that of sodium is very small. If the husks therefore possess photosynthetic systems near their peripheral surface only, then either the large concentrations of sodium or potassium are only in deeper layers, or else such a photosynthetic system can function in

the presence of large concentrations of salts. Later tables consider these salts of the walnut husk, their solubility, the influence of sunlight on their composition, their organic combination, and other factors of interest in this connection.

Effect of shading on composition of walnut fruits

The effect of sunlight on the ash composition of walnut husks and kernels is seen in table IX. Some of the nuts attached to the tree were covered when about six weeks old with heavy brown paper bags, which excluded much but not all of the light. Both the control and the bagged walnuts were on the same tree at Irvine, California, and were removed on September 18. Table IX shows that the dry matter of the husks in full sunlight contained more ash than when given partial sunlight. It is not unlikely that this increase of ash in the husks of walnuts exposed to full sunlight may become so great in the interior districts of California, where high temperatures in late summer may prevail, that injurious concentrations of ash may bring about the burning, to the point of destruction or at least unmarketability of the nut. The percentages of sodium, potassium, and phosphorus in the ash were somewhat less, but the percentage of calcium was greater when the husks were given full than when given partial sunlight. The dry matter of the husks showed a greater concentration of all the determined ash constituents, except phosphorus, when in full than when in partial sunlight, possibly due to a great transpiration rate. The sunlight had but little effect on the total ash content of the dry kernels. Those from the walnuts in full sunlight had the greater percentages of sodium, potassium, and calcium, whether calculated in terms of the ash or of the dry matter. The husks and their kernels both contained less calcium when given partial sunlight than when given full sunlight. Irving and Becking (5) have shown that corallines use up more calcium in the light than in darkness. It is of interest to find (table IX) that, accompanying the reduced calcium in the kernels from nuts given partial sunlight, there is a considerable increase in the concentration of magnesium. It is also of interest that the sum of the magnesium of the husks and kernels in 100 gm. dry matter is about the same after exposure to full sunlight as after exposure to partial sunlight only. This type of relation-

ship does not hold for any of the other bases. It might be construed to mean that less magnesium is required in partial sunlight by the photosynthetic processes, the kernel appropriating the portion not required by the husk. It also indicates that magnesium absorption may be independent of the action of full sunlight, since the total magnesium is about the same in the two cases.

TABLE IX

EFFECT OF SHADING ON ASH COMPOSITION OF WALNUT HUSKS AND KERNELS WHILE ATTACHED TO TREE

	Exposed to sunlight		Exposed to sunlight until about six weeks old, then covered with heavy brown paper bags	
	Husks	Kernels	Husks	Kernels
Ash as percentage of dry matter	22.11	2.75	18.19	2.82
Ash constituents (percentage of ash)				
Na	10.12	2.86	11.94	1.57
K	44.65	22.09	47.47	17.24
Ca	3.89	4.42	2.49	3.19
Mg	1.11	3.16	0.83	6.83
Cl	13.71	1.29	12.53	1.28
SO_4	1.46	0.64	1.59	0.40
PO_4	3.57	40.28	5.39	39.40
Grams in 100 gm. of dry matter (from ash analyses)				
Na	2.2375	0.0787	2.1719	0.0443
K	9.8721	0.6075	8.6348	0.4862
Ca	0.8601	0.1216	0.4529	0.0900
Mg	0.2454	0.0869	0.1510	0.1926
Cl	3.0313	0.0355	2.2792	0.0361
SO_4	0.3228	0.0176	0.2892	0.0113
PO_4	0.7893	1.1077	0.9804	1.1111

ASH OF WALNUT LEAVES AFFECTED BY ACCUMULATION OF CHLORINE

An examination of walnut trees under field conditions where the soil contains considerable alkali salts shows that considerable amounts of chlorine may be absorbed. When the accumulation of chlorine becomes excessive as the leaf matures, marginal and tip burning results and the leaves may fall prematurely. The trees in some walnut districts may have chlorine burn without other complicating factors, such as excessive concentration of various other alkali

salts. In these cases the soil may contain large amounts of soluble calcium, and under such conditions a given concentration of chlorine may be less injurious than when less favorable bases are in preponderance. Table X gives data in regard to the chlorine content of the ash of leaves obtained from walnut trees of this nature.

TABLE X

COMPOSITION OF ASH OF WALNUT LEAVES AFFECTED BY CHLORINE

	Leaves from trees in saline soil, Irvine, Calif., June 5, 1925	Leaves from trees surrounded by salt-injured trees, no injury as yet, Irvine, June 22, 1925	Salty soil near drainage ditch, leaves burned, Irvine, June 22, 1925	Salt burn, Irvine, July 13, 1925	Leaf burn, Irvine, Sept. 18, 1925	Good leaves, Irvine, Sept. 18, 1925 (1). Good leaves, Anaheim, Calif., Sept. 18, 1925 (2)	Marginal burning, Ventura, Calif., Sept. 22, 1925	Marginal burning, nearly all leaves prematurely abscised for [illegible] Calif., Season, Santa Barbara, Calif., Sept. 19, 1925
Ash as percentage of dry matter.....	10.19	9.90	8.47	10.26	11.55	12.75(1)	12.67	11.81
Ash constituents (percentage of ash)								
Na.....	5.33	3.43	5.10	4.53	2.32	3.02(1)	1.73	4.89
K.....	19.13	12.24	15.95	16.26	6.78	12.54(1)	7.27	15.86
Ca.....	17.67	23.58	19.75	18.60	20.09	23.13(1)	26.53	18.60
Mg.....	6.05	6.76	7.46	7.64	9.11	6.70(1)	6.03	6.11
Cl.....	13.34	8.50	17.78	14.89	9.47	4.54(1) 1.58(2)	7.25	13.31
SO_4.....	2.42	2.10	3.28	2.57	2.73	2.42(1) 3.85(2)	2.79	4.41
PO_4.....	12.31	8.06	12.00	8.57	11.82	4.28(1)	4.92	6.90

The sodium content of the ash of these leaves is never high, and the calcium content is like that of normal leaves of the same age. It may be noted that good walnut leaves collected as late as September 18 have been found to contain from 1.5 to 5 per cent of chlorine in their ash without visible injury, although the larger amount may not be conducive to the best growth the trees might make, were the chlorine content lower. Where the soil solution is rich in chlorine, walnut leaves may contain excessive amounts. The values given in table X represent only the non-volatile chlorine, and these alone are

extremely high in some cases. It will be seen that the sulphate content of the ash of walnut leaves in table X is very uniform, although many of the soils in which the trees were growing are moderately rich in sulphate as well as in chlorine. The sulphate of calcium is not as soluble as the chloride. The reason for the low concentration of sulphate, although not for its uniformity, is the fact that much of the sulphur in walnut leaves is present in the organic form. Reference will be made to the organic chlorine and sulphur in a later connection.

The sensitivity of walnut trees to salts is seen in experiments in which walnut trees were grown in soil in large pipes 5–6 feet high and about 2 feet in diameter. As the soil was being placed in the pipes, a layer of one of the calcium salts about 0.25 inch deep was placed in the soil about one-fourth of the distance down from the top of the soil. This was in imitation of strata of salts that may possibly occur in soils, since it is difficult otherwise to explain why trees may suddenly be injured, or may suddenly change from poor to good growth.

A single layer was made in each pipe, calcium hydrate, tricalcium phosphate, and calcium carbonate being used for different pipes. After making excellent growth early in the season, the trees began to show bad leaf burn without showing more calcium or magnesium in the leaves than occurs in good walnut leaves of the same age, while the trees in untreated soil showed excellent growth throughout the experiment. In such cases the salts probably act by hindering absorption of water by the roots, rather than by producing excessive salt concentrations in the leaves.

COMPARATIVE CHLORINE ACCUMULATIONS IN DECIDUOUS-LEAVED TREES

Other deciduous-leaved trees are also sensitive to chlorine accumulation, and it may be well to consider some others in comparison with the walnut. Table XI shows the increased amounts of chlorine in injured persimmon leaves collected in the autumn. The leaves were all mature, and instead of showing the autumnal colors typical of this species, they began to burn and abscis, and many of the fruits fell before they were ready to be picked. The chlorine in the

soil was usually accompanied by abundant calcium, and simply leaching the soil to carry down the excess chlorine was sufficient to bring the trees back to an excellent condition the following year. The persimmon trees were interplanted with grapefruit trees which showed no unfavorable symptoms. It is seen in table XI that about half of the chlorine, sulphur, and phosphorus exists in the organic form. Deciduous trees differ from citrus in their behavior as regards

TABLE XI

EFFECT OF ALKALI SALTS ON COMPOSITION OF PERSIMMON LEAVES

	Good leaves, La Habra, Calif.	Good leaves, Tustin, Calif.	Leaves just beginning to show dried spots and marginal burn, Orange	Leaves burned along margins and between veins, Orange	Badly burned leaves, Orange, Calif.
Ash as percentage of dry matter	14.86	15.43	16.17	14.88	16.53
Ash constituents (percentage of ash)					
Na	1.50	1.98	2.05	2.69	1.94
K	9.94	9.20	9.67	11.36	8.79
Ca	24.75	24.70	25.00	22.94	24.82
Mg	6.83	6.87	6.86	4.73	6.34
Cl	1.80	2.89	4.25	6.88	7.90
SO_4	2.52	3.56	2.61	3.47	2.81
PO_4	3.99	3.82	3.85	4.22	4.23
Percentage of dry matter					
Total Cl	0.54			1.70	2.22
Cl of ash	0.27			1.02	1.31
Total S as SO_4	0.71			0.95	0.88
SO_4 of ash	0.38			0.52	0.47
Total P as PO_4	0.68				
PO_4 of ash	0.59				

absorption of chlorine or sulphur, in that they lose much of their accumulation by means of leaf abscission each year, whereas citrus trees must go on adding to the accumulation over a period of years. The table shows the small percentage of sodium in the ash and the relatively large concentration of divalent in comparison with monovalent bases.

A comparison of the deciduous walnut tree with the evergreen citrus tree as regards the organic combination of the accumulated chlorine, sulphur, and phosphorus, is shown in table XII. When

several samples from trees have been taken in a given locality they are designated by numbers in the table. The ash of lemon fruit may contain all of the chlorine and much of the sulphur when the amount present is small, but when comparatively large amounts are present much may be in the organic form. Most of the phosphorus in lemon fruit is retained upon ashing at low temperatures. Much of the chlorine and sulphur of citrus leaves containing large amounts of these constituents is lost when the dry matter is ashed, presumably because they are in organic combination. Most of the sulphur of walnut shoots is in organic form, while the phosphorus is almost entirely retained in the ash. As much as one-half of the chlorine of walnut leaves may be lost in ashing the dry matter at low temperatures, while the organically combined sulphur and phosphorus may be considerable. In walnut leaves taken from sand cultures in which calcium sulphate was mixed with the sand, and the sand given Hoagland's solution lacking calcium, the dry matter of the leaves showed 2.40 per cent total S as SO_4, while normal leaves contained 0.99 per cent. Practically all of the chlorine of walnut husks in table XII is retained upon ashing the dry matter, as is most of the sulphur and phosphorus.

Absorption from solutions of sodium chloride and sodium sulphate by growing fruits

If the shoots attached to walnuts are placed into solutions such as distilled water, N/100 sodium chloride, or N/100 sodium sulphate, and then the whole nuts, husks, and nuts with husk removed are analyzed, it is found that an appreciable gain in concentration in sodium has been made only in the nuts with husks removed (table XIII). In other words, the kernel gained largely in sodium content but the husk very little if at all. In the sodium chloride experiments practically no change took place in the chlorine content of the ash, but a gain in the total chlorine took place in the dry matter of the husks and kernels. The sulphur content of the ash of the husks greatly increased in the sodium sulphate solutions, although that of the kernels showed no appreciable increase. Sodium chloride solutions increased the organic chlorine in the husks and kernels, while sodium sulphate solutions increased the organic sulphur in both

TABLE XII

TOTAL CHLORINE, SULPHUR, AND PHOSPHORUS CONTENT OF DRY MATTER OF CITRUS AND WALNUT TREES COMPARED WITH CONTENT OF THESE CONSTITUENTS IN THE ASH

	TREE-RIPE LEMON FRUIT, PLOT U, RUBIDOUX TRACT	TREE-RIPE LEMON FRUIT FROM TREES AFFECTED BY CHLORINE SALTS, IRVINE	TREE-RIPE LEMON FRUIT AFFECTED BY SULPHATE IN IRRIGATION WATER, VENTURA	MATURE LEMON LEAVES PLOT U, RUBIDOUX TRACT	MATURE LEMON LEAVES AFFECTED BY SULPHATE IN IRRIGATION WATER, VENTURA	MATURE VALENCIA ORANGE LEAVES, EASILY ABSCISSED, RIVERSIDE	LAST MATURE CYCLE OF GOOD PLACENTIA WALNUT SHOOTS, BOX SPRINGS	GOOD MATURE WALNUT LEAVES, RIVERSIDE	MATURE WALNUT LEAVES, BADLY BURNED, IRVINE	WALNUT LEAVES AFFECTED BY SALT BURN, SANTA BARBARA	BURNED HUSKS FROM WALNUT TREES AFFECTED WITH "YELLOWS," IRVINE
Percentage of dry matter									2.21(1)	3.04(1)	2.25(1)
Total Cl	0.04	0.45	.....	.....	.....	3.58	0.02	.....	1.48(2)	1.94(2)	
									2.41(3)		
Cl of ash	0.04	0.34	.....	.....	.....	2.52	0.01	.....	1.09(1)	1.57(1)	2.21(1)
									1.12(2)	0.92(2)	
									1.39(3)		
Total S as SO_4	0.20	0.21	0.54	0.90	1.80(1)	.....	0.24	1.00	0.73(1)	.....	1.03(1)
					2.42(2)				.42(2)	0.76(2)	
					3.14(3)						
SO_4 of ash	0.15	0.16	0.16	0.45	1.26(1)	.....	0.05	0.45	0.32(1)	.....	0.94(1)
					1.85(2)				0.28(2)	0.35(2)	
					2.52(3)						
Total P as PO_4	0.72	0.77	.....	.....	.....	.....	0.64	.....	1.88(1)	.....	1.89(1)
PO_4 of ash	0.60	0.67	.....	.....	.....	.....	0.60	.....	1.37(1)	0.82(1)	1.80(1)
									1.20(2)	0.62(2)	

husks and kernels. Absorption of inorganic salts from such solutions by walnuts through attached shoots was accompanied by increases

TABLE XIII

EFFECT OF SALT SOLUTIONS ON COMPOSITION OF WALNUTS ON SHOOTS PLACED IN SOLUTION

SHOOTS 4"–6" LONG WITH TERMINAL NUTS; BASE OF SHOOT PLACED IN SOLUTION	DISTILLED WATER			N/100 NaCl			N/100 Na_2SO_4		
	Whole nuts (husk, shell, kernel)	Husks	Nuts with husk removed	Whole nuts (husk, shell, kernel)	Husks	Nuts with husk removed	Whole nuts (husk, shell, kernel)	Husks	Nuts with husk removed
Ash as percentage of dry matter	5.79	6.09	6.29	6.38	5.69	6.10	5.95	5.78	6.26
Ash constituents (percentage of ash)									
Na	7.22	9.09	6.40	7.00	9.73	13.26	9.34	8.96	10.30
K	37.49	30.15	40.89	33.35	33.21	39.43	35.86	28.35	40.87
Ca	9.32	11.80	5.39	10.29	9.45	4.96	8.14	11.30	5.25
Mg	3.21	3.78	4.43	3.90	3.67	3.90	3.38	4.08	4.16
Cl	1.93	1.63	2.66	2.01	1.92	2.92	1.62	1.52	3.03
SO_4	4.28	4.80	2.45	3.97	4.65	3.01	7.99	12.38	3.20
PO_4	14.89	11.70	19.94	13.01	11.85	18.67	15.20	11.14	16.96
Percentage of dry matter									
Cl	.....	0.11	0.14	.....	0.24	0.27	.....	.....	.....
SO_4	.....	0.22	0.18	.....	.....	.....	.....	0.38	0.44

TABLE XIV

EFFECT OF CHLORIDES UPON ASH COMPOSITION OF WALNUT HUSKS

	GOOD WALNUT HUSKS, IRVINE, SEPT. 18, 1925	WALNUT HUSKS FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925	WALNUT HUSKS SPOTTED AND BURNED, SALT ON HUSKS, ALKALI SOIL, IRVINE, SEPT. 18, 1925
Ash as percentage of dry matter	22.99	20.62	16.75
Ash constituents (percentage of ash)			
Na	10.82	10.09	10.62
K	44.94	40.76	41.92
Ca	4.86	4.09	4.56
Mg	1.62	1.24	2.00
Cl	3.53	15.86	13.18
SO_4	2.46	2.37	5.61
PO_4	1.45	5.86	10.75

in chlorine or sulphur, some of which were almost entirely of organic nature. Such experiments were carried on with frequent changes of

solution during early June, when the kernel contents were still in a more or less liquid or partially jelled condition.

If the chlorine is allowed to accumulate in walnut husks slowly over a long period in the field, however, and the samples gathered for analysis when the monovalent and divalent bases of the husks are high, the ash of the husks may be found to contain large amounts of chlorine (table XIV). In contrast with the low amount of chlorine in the husks of good walnuts, walnut husks may accumulate so much

FIG. 3.—Walnuts of different ages showing extreme effects of chloride accumulation upon husks. Chemical tests of light-colored incrustations in sunken areas indicate accumulations of chloride.

chlorine that it can be gently scraped off the surface with a scalpel onto a slide (fig. 3), and the test for chlorine obtained with silver nitrate.

SODIUM AND POTASSIUM IN LEAVES AND FRUITS

If various tree materials are ashed in the presence of sulphuric acid, to prevent volatilization of the alkali bases, it is found that the bases of the dry matter are practically non-volatile (table XV). This is of great interest, in that table XVI shows the large concentration of bases of which potassium is outstanding in amount. The results given in table XVI represent the average of three closely agree-

ing analyses of six entire husks that had not touched the ground. It still remains to be determined whether the bases are combined with silicates, carbonates, bicarbonates, nitrates, etc., as the chlorine content of these husks is known to be low, and the reaction values of basic and acidic constituents do not approach a balance.

TABLE XV

COMPARISON OF TOTAL SODIUM AND POTASSIUM OF DRY MATTER WITH THAT FOUND IN ASH OF TREES

MATERIAL	No H_2SO_4 USED IN ASHING DRY MATTER		H_2SO_4 USED IN ASHING DRY MATTER	
	Percentage of dry matter			
	Na	K	Na	K
Valencia orange leaves from trees in soil given excessive potassium	0.14	5.90	.....	5.95
Good Placentia walnut leaves	0.07	1.33	0.07	1.34
Good Placentia walnut husks	.....	10.34	.....	10.54
Good Placentia walnut kernels	0.04	0.43	0.07	0.44
Persimmon leaves	0.10	.....	0.11	.....

TABLE XVI

COMPOSITION OF ASH OF EUREKA WALNUT HUSKS FROM TREES AT BOX SPRINGS TRACT, OCTOBER 20, 1926

		ASH AS PERCENTAGE OF DRY MATTER	PERCENTAGE OF ASH					
			Na	K	Ca	Mg	SO_4	PO_4
Fresh weight	Dry weight	15.82	10.69	43.08	6.88	1.66	1.97	4.00
		Grams per husk (from ash analyses)						
23.66	3.25	0.5136	0.0549	0.2211	0.0354	0.0086	0.0101	0.0205

SEASONAL CHANGES IN ASH CONSTITUENTS OF YOUNG WALNUT FRUITS

If the composition of the ash of good walnut fruits of varying ages is studied (table XVII), it is found that the ash of the dry matter of the kernels decreases with age, because the increase in the weight of the dry matter is more rapid than that of the increase in

TABLE XVII

CHANGES IN COMPOSITION OF ASH OF GOOD WALNUT FRUITS OF DIFFERENT AGES

	MAY 26, 1925			JUNE 3, 1925			JUNE 9, 1925			JUNE 22, 1925			AUGUST 14, 1925		
	Husks	Whole nuts (husk, shell, kernel)	Nuts with husks removed	Husks	Whole nuts (husk, shell, kernel)	Nuts with husks removed	Husks	Whole nuts (husk, shell, kernel)	Nuts with husks removed	Husks	Whole nuts (husk, shell, kernel)	Nuts with husks removed	Husks	Whole nuts (husk, shell, kernel)	Nuts with husks removed
Ash as percentage of dry matter	7.03	6.68	6.53	6.18	5.86	6.09	6.05	5.49	5.47	6.70	5.10	3.16	12.42	4.39	1.76
Ash constituents (percentage of ash)															
Na	8.11	8.63	11.28	7.55	9.76	9.63	8.65	9.53	12.63	10.85	12.19	10.80	11.42	7.61	3.59
K	29.51	33.09	40.32	33.20	35.19	39.19	35.08	35.60	40.24	39.00	37.62	39.57	46.06	42.48	30.65
Ca	13.10	10.30	4.32	10.19	8.42	4.92	10.07	8.24	4.93	7.34	7.18	6.17	4.40	6.84	9.00
Mg	4.88	4.07	3.48	4.12	3.90	3.39	3.87	3.86	3.82	3.14	3.92	3.70	1.93	3.21	6.06
Cl	2.08	1.84	1.45	2.28	2.23	1.71	1.81	1.99	1.58	1.98	1.59	1.57	3.26	3.55	2.14
SO_4	5.86	4.53	3.50	5.03	4.11	2.77	5.31	4.50	3.61	4.70	3.67	2.35	2.54	2.49	1.05
PO_4	11.47	15.52	19.81	9.18	13.62	19.05	9.46	13.84	20.61	8.47	12.55	23.37	2.71	9.63	31.06
Fresh weight (gm.)	228	(25)235	217	(25)488	(25)496	542	269	(25)621	.....	(20)320	(20)653	(20)295	(8)198	(8)343	(8)160
Dry weight (gm.)	32	30	21	75	59	49	41	76	.....	42	97	48	24	90	73

the ash content (HAAS and BATCHELOR 1). During July and August the husks greatly increase in ash content, due in part no doubt to increased transpiration. The percentages of sodium and potassium in the ash of the husks increase with their age, while those of calcium and magnesium decrease. The amount of sodium and potassium, and that of calcium and magnesium, in the dry matter of the walnuts with husks removed, decreases with advancing age of the nuts, owing to the decrease in the percentage of ash. Sodium and potassium greatly increase, and the calcium decreases in the ash of walnut husks at a time when the leaves are increasing the percentage of calcium in their ash. The percentage of potash in the ash of walnut leaves decreases, while that of calcium increases with increasing age of the leaves. This is just the opposite of what happens in walnut husks.

In view of the extremely high amount of bases in the walnut husk, the following questions may be asked, although at present we cannot answer them: (1) Does the large amount of bases continue to increase until the death and splitting open of the husk follows as a consequence? (2) Is the walnut husk a dumping ground or an outlet for surplus absorption of bases? (3) Does the production of walnut fruits depend on such a surplus of bases in the husks? These questions indicate but a few of the many problems that have arisen.

EFFECT OF SALINE IRRIGATION WATER ON WATER SOLUBILITY OF INORGANIC CONSTITUENTS OF DRY MATTER OF WALNUT HUSKS

It is of interest, in connection with the large concentration of bases in walnut husks, to study the water solubility of the husks of young and of nearly mature walnuts, and to observe the changes that take place in this solubility when husks are taken from trees injured as a result of salinity of the soil. Table XVIII gives data which show that the ash of the husks from trees in saline soil may be less than that from trees in good soil. The ash of the water-soluble fraction as a percentage of the total ash increases with the age of the husks. The total calcium, as a percentage of the total ash, also decreases with increasing age of the husks. There is a larger percentage of the total calcium in the ash of the water-soluble fraction when

TABLE XVIII

EFFECT OF ABSORBED SALTS ON WATER-SOLUBILITY OF INORGANIC CONSTITUENTS OF DRY MATTER OF WALNUT HUSKS

	Husks of young walnuts from salt-injured trees, Irvine, June 3, 1925			Husks of young walnuts from good trees, Irvine, June 22, 1925			Husks of walnuts from trees 16–18 in soil receiving saline irrigation water, Sept. 9, 1925			Husks of walnuts from good trees, Irvine, August 15, 1925		
	Soluble fraction %	Insoluble fraction %	Percentage	Soluble fraction %	Insoluble fraction %	Percentage	Soluble fraction %	Insoluble fraction %	Percentage	Soluble fraction %	Insoluble fraction %	Percentage
Ash of different fractions as percentage of dry matter	3.49	1.34	.....	5.51	1.58	.....	8.77	0.84	.....	10.46	2.26	.....
Ash of soluble fraction as percentage of ash	72.31	.....	.....	77.68	.....	.....	91.26	.....	.....	82.22	.....	.....
Total Ca as percentage of total ash	.....	.....	11.49	.....	.....	7.95	.....	.....	2.31	.....	.....	5.37
Percentage of total Ca in each fraction	18.41	81.59	.....	4.96	95.04	.....	19.37	80.63	.....	4.10	95.90	.....
Ca of each fraction as percentage of ash of same fraction	2.93	33.86	.....	0.51	33.84	.....	0.49	21.31	.....	0.27	28.97	.....
Percentage of total Mg in each fraction	78.06	21.94	.....	75.90	24.10	.....	76.07	23.93	.....	70.83	29.17	.....
Total Mg as percentage of total ash	.....	.....	4.06	.....	.....	2.75	.....	.....	2.43	.....	.....	1.88
Percentage of total Na in each fraction	94.20	5.80	.....	81.70	18.30	.....	68.21	31.79	.....	56.49	43.51	.....
Total Na as percentage of total ash	.....	.....	1.43	.....	.....	2.15	.....	.....	2.03	.....	.....	1.03
Percentage of total K in each fraction	97.01	2.99	.....	96.75	3.25	.....	97.50	2.50	.....	95.31	4.69	.....
Total K as percentage of total ash	.....	.....	29.14	.....	.....	35.53	.....	.....	45.31	.....	.....	43.40

the husks are from salt-injured trees than when they are from good trees, regardless of age as a factor. The soluble calcium as percentage of the ash of the soluble fraction is greatest in each case when the husks are from salt-injured trees. There is more total magnesium as percentage of the total ash and a greater percentage of the total magnesium, sodium, and potassium in the water-soluble form in the husks from salt-injured trees, than in those from good trees. The concentration of potassium increases with age, and over 95 per cent of it is water-soluble.

Juice of walnut husks and young kernels

Analysis of the ash of the juice of walnut husks (table XIX) shows a large concentration of some of the soluble salts, and indicates a low concentration of divalent bases in comparison with the

TABLE XIX

Composition of juice of walnut husks and that of young walnut kernels

Material	Condition of trees	Collected	Sample (cc.)	pH	Parts per million							
					Ash	Na	K	Ca	Mg	Cl	SO_4	PO_4
Juice of walnut husks	Normal	June 23, 1926	100	....	6881	868	2713	76	306	....	537	1254
Juice of young walnut kernels	Salt affected	May 25, 1925	14	5.3	.....	500	4184	440	....	....	....	....
	Salt affected	May 25, 1925	12	5.4	.....	702	3741	383	....	....	....	....
	Salt affected	June 22, 1925	28	5.2	10900	1694	4826	350	207	43	162	1173
	Salt affected	June 22, 1925	10	5.2	10700	2490	4494	460	320	60	74	1490
	Normal	June 22, 1925	45	....	.....	1167	3758	269	177	....	....	....

monovalent ones. A high concentration of phosphate is also evident. By careful manipulation, it was possible to remove kernels from walnuts while still in the liquid condition, and then secure the liquid by puncturing the kernel. The 10 cc. and 28 cc. samples were ashed and the ash was analyzed; whereas in the other samples direct aliquots of the juice were made for analysis of the various constituents. It was not possible to determine chlorine directly in the fresh juice, because of the reducing action of the juice on silver nitrate.

The juice of kernels obtained at Irvine, California, always gave pH values close to 5.2, which is the same as that found by Hoagland and Davis (4) for the sap of *Nitella* cells. In *Nitella* sap, as compared

with walnut kernel liquid, the monovalent bases are lower, the divalent bases about the same, the phosphate lower, and the chlorine and sulphur higher. The specific conductivity of the juice obtained from walnut kernels collected June 21 correspond at 25° C. approximately to that of an N/10 solution of potassium chloride. Titration of the juice with N/10 NaOH, with phenolphthalein as indicator, showed that its acidity corresponded approximately to an N/40 acid. Table XIX shows the large concentrations of the constituents of the clear, colorless juice of the young kernels. This juice from salt-affected trees contained larger concentrations of sodium, potassium, calcium,

TABLE XX

TOTAL S AND P OF WALNUT KERNEL JUICE AND EXPRESSED JUICE OF WALNUT HUSKS (JUNE 22, 1926)

	S (GM.)	P (GM.)
15 cc. walnut kernel liquid with $Mg(NO_3)_2$ added	0.0023	0.0026
15 cc. walnut kernel liquid without $Mg(NO_3)_2$ added	0.0009	0.0025
10 cc. juice (extracted by crushing husks of good walnuts) with $Mg(NO_3)_2$ added	0.0016	0.0019
10 cc. juice as preceding but no $Mg(NO_3)_2$ added	0.0016	0.0015

and magnesium than that from normal trees. The sodium of the kernels from the salt-affected trees showed a marked increase with increasing age of the kernel. The changes which the constituents of the kernels undergo in their later development deserve study.

The volatility of sulphur and phosphorus upon ashing the juice of husks and of young kernels still in the liquid condition is shown in table XX. In the kernel juice the sulphur was largely volatile upon ignition, whereas in the husk juice none was lost in this way. Very little of the phosphorus was lost during the ashing process.

VOLATILITY OF SULPHUR AND PHOSPHORUS OF WALNUT KERNELS ON ASHING

The great loss of sulphur that occurs when mature kernels are ashed is seen in table XXI. The loss in phosphorus upon ignition is also large, but the percentage loss is much less than that of sulphur. Much of the sulphur and phosphorus of walnut kernels exists in organic combination and is easily volatilized upon ignition.

EFFECT OF ALKALI SALTS ON COMPOSITION OF WALNUT KERNELS

The effect of alkali salts on the composition of walnut kernels is seen in table XXII. The dry matter of kernels from nuts of trees in saline soil had a higher ash content than that from trees on good

TABLE XXI

TOTAL SULPHUR AND PHOSPHORUS CONTENT OF MATURE WALNUT KERNELS

LOCALITY	CONDITION OF TREES	GRAMS IN 100 GM. OF DRY MATTER			
		SO_4 from ash analyses	SO_4 from total S analyses	PO_4 from ash analyses	PO_4 from total P analyses
Irvine	Good	0.018	0.680	1.110	2.273
Trees 13–15, Riverside	Good	0.017	0.628	1.095	1.865
Irvine	Good	0.011	0.640	1.105	1.718
Irvine	Badly affected by alkali	0.024	0.722	1.085	2.035

TABLE XXII

EFFECT OF ALKALI SALTS ON COMPOSITION OF WALNUT KERNELS

	KERNELS FROM GOOD WALNUTS, IRVINE, SEPT. 18, 1925	KERNELS FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925	KERNELS FROM HUSKS WITH BURNED AREAS AND CHLORIDE ON SURFACE, FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925
Ash as percentage of dry matter	(2.07)	(2.75)	(3.39)
Ash constituents (percentage of ash)			
Na	4.60	4.04	3.98
K	25.41	26.57	30.44
Ca	4.19	3.74	5.10
Mg	5.20	5.96	9.47
Cl	0.51	2.08	3.94
SO_4	0.49	0.86	2.47
PO_4	53.29	39.36	49.18
Fresh weight (gm.)	86.0	130.0	78.0
Dry weight (gm.)	64.0	90.0	35.0

soil, owing to the fact that kernels from trees on saline soil have a lower content of dry matter than those of trees on good soil. The percentages of each of the bases in the dry matter, as calculated from the ash analyses of table XXI, are higher in the kernels from the trees on saline soil. The percentage of chlorine and sulphate in the ash is greatest for the trees on saline soil.

FURTHER INVESTIGATION

Very little is known about the seasonal or the cyclic changes in the inorganic relationship in fruit trees, although much is known regarding the migration of constituents of leaves during senility and just prior to abscission. The nature of the balance between the inorganic constituents of the leaves and of the fruit has received very little attention heretofore, investigations having concerned themselves largely with the carbohydrate-nitrogen ratio. While there is need of further study on the inorganic physiology of healthy walnut trees, the present studies have made some effort in this direction, and have also compared such data in many cases with the situation that arises when there are unusual salt effects of the environment upon the inorganic constituents, growth, and behavior of the trees.

Summary

1. The effects of saline irrigation water upon walnut trees have been studied with reference to the effects upon behavior and growth, and upon the relationship between the inorganic constituents.

2. The continued application of saline irrigation water, to tanks of soil planted to walnut trees, has resulted in a reduced size of the leaves with occasional leaf burn, but no indications of mottle leaf or of walnut "yellows."

3. There exists a gradient in the ash content of the dry matter of walnut trees, which in good soil has its minimum in the rootlets but in saline soil has a minimum near the level of the surface of the soil.

4. The production of a new cycle of growth brings about changes in the relationships of the inorganic constituents of the water-soluble and insoluble fractions of the dry matter.

5. Although the sodium, potassium, and calcium contents of the drainage water of artificially made saline soil, in which walnut trees were grown, were much greater than the magnesium content, yet the woody portion and leaves of the trees contained less sodium, potassium, and calcium but more magnesium than the corresponding parts of control trees in good soil.

6. Walnut trees may absorb large amounts of chlorine and sul-

phur, much of which may be in organic combination. Persimmon leaves, and citrus leaves and fruit, as well as walnut leaves and fruit have been shown to be able to absorb large amounts of chlorine and sulphur, and to place much of it in organic combinations. The dry matter of leaves of walnut trees growing in soils rich in chlorine may lose one-half or more of its chlorine upon gentle ignition, whereas practically none is lost in the case of badly burned mature walnut husks.

7. Saline soils affect not only the inorganic constituents of the trunk, shoots, and leaves, but also those of the husk and kernel.

8. The effects of the artificially made saline soil upon walnut trees and their fruit are not confined alone to accumulations of chlorine or sulphur, but involve equally important changes in the relationships between the bases within the tissues.

9. When a soil was artificially made saline in controlled tank cultures of walnut trees, the effect of salinity of the soil solution was in the direction of reducing rather than increasing the ash content in the portions of the plant above the soil level, as compared with homologous portions of suitable control trees.

10. The effect of direct sunlight upon walnuts is to bring about an increase in the ash constituents of the dry matter. Such effects, when extreme temperatures also occur, may be so destructive in their action as to cause burning of the husks with its consequent effects upon the marketability of the nuts.

11. Absorption from salt solutions by short shoots with walnuts attached, while the kernels were still in the liquid condition, resulted in a considerable increase in sodium in the kernel portion. Although a considerable amount of chlorine and sulphur was absorbed by the shoots into the walnuts, only the increased sulphur of the husks was retained upon ignition.

12. The ash content of walnut husks increases most rapidly from July to late summer. Mature husks are extremely rich in bases, but the importance of such high concentrations in the physiology of the husk is very little understood. The water solubility of the inorganic constituents of husks changes considerably according to the nature of the soil solution bathing the tree roots.

13. The pH of the juice of young walnut kernels still in the liquid condition was about 5.2. Such juice was extremely rich in inorganic constituents.

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CYTOLOGY AND LIFE HISTORY OF VAUCHERIA GEMINATA

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(WITH PLATES XIII, XIV)

Introduction

It is generally agreed that the oogonium of *Vaucheria* is multinucleate when young, and in its mature stage uninucleate; but there is a difference of opinion concerning what happens to the numerous nuclei that occur in the young oogonium as it develops from the multinucleate into the uninucleate or mature condition.

Conflicting accounts of oogenesis in *Vaucheria* have been given on the one hand by OLTMANNS (6) and on the other by DAVIS (2). OLTMANNS found that all the nuclei except one withdraw from the oogonium of *V. sessilis* before the formation of the membrane which separates the oogonium from the parent filament. DAVIS contended that the nuclei remain in the oogonium of *V. geminata* until after the formation of the membrane, and that they begin to disintegrate just before the formation of the cross wall and continue to disintegrate very rapidly after its formation, until, by the time the cross wall is completely developed, the nuclei are hardly recognizable. OLTMANNS' results were confirmed by HEIDINGER (4). In this respect DAVIS pointed out a resemblance in the behavior of the nuclei in gametogenesis in *Vaucheria* and in *Saprolegnia*, several members of the Peronosporales, and certain of the Ascomycetes.

DAVIS (2) also stated that the nuclei are carried into the young oogonium by the inflow of protoplasm. He found that their number varies from twenty to fifty. Furthermore, he stated that it is probable that mitotic figures are present in the vegetative branch and also in the earlier stages of the young oogonium, but he was unable to see the spindles. He concluded that there are no mitoses during the growth of the oogonium and none after separation by the cross wall. DAVIS suggested this as one of the points in which this process differs from that found in *Saprolegnia* and the Perono-

sporales, where there seem to be always one or two general mitoses after the oogonium is formed.

KURSSANOW (5) showed that mitosis, or nuclear division, occurs in the filament of *Vaucheria*, and that the number of chromosomes is rather small.

Material and methods

The first collection of the material from which most of the cytological results were obtained was made in October, 1924, from the banks of the Rivanna River near the Woolen Mills, Charlottesville, Virginia. For comparison, collections were made in the neighborhood of the University of Virginia, and from greenhouses. The first collection showed a high degree of reproductive activity, with numerous oogonia and antheridia in practically every stage of development. The species studied was *V. geminata*.

Some of the material was fixed in the field immediately upon collection. A second lot was brought into the laboratory, kept moist with as little disturbance as possible until 2:30 A.M. the following day, and then fixed. There were about 12 hours between the fixation of the first lot and that of the second. The fixing fluid consisted of sixteen parts of 1 per cent chromic acid and one part of glacial acetic acid. This was mixed with formalin, two parts of the first mixture to one of the formalin. To this fluid about 1 cc. of 1 per cent osmic acid was added to every 100 cc. of chromo-aceto-formalin mixture. The formalin and osmic acid were mixed with the chromic and acetic acids just before using.

The material remained in the fixing fluid for about 30 minutes. It was washed 4 hours in running water, then carried to 100 per cent alcohol by very slow changes in the weak alcohols, beginning with 2.5, 5, and 8 per cent, etc. The material remained at least 2 hours in each strength of alcohol, and was then imbedded in paraffin by gradual transfer through xylol and paraffin solutions, then sectioned. Sections were cut 5 μ and 7 μ thick.

Of a number of stains experimented with, safranin and gentian violet in 1 per cent solution gave the best results. Both resting nuclei and mitotic stages in the oogonium were excellently stained. The safranin in 50 per cent alcohol was first applied to the slide for 2 or 3 minutes, then gentian violet in 25 per cent alcohol was applied

for 2 or 3 minutes, and the slide quickly rinsed in 95 and 100 per cent alcohols. Clove oil was used for differentiation while the specimen was observed under a 4 mm. objective. This stain gave very satisfactory results in all stages of development of the oogonium.

Safranin in 50 per cent alcohol and light green in clove oil gave best results when the stains were used in terms of minutes and seconds rather than hours; however, this combination was not satisfactory except in occasional instances. Haidenhain's haematoxylin was not satisfactory because the chromatophores took the stain so deeply that the nuclei could not be differentiated and distinguished from them. Acid fuchsin and methyl green were used according to the schedule for safranin and gentian violet. This combination gave results almost as good as the latter. Delafield's haematoxylin used for 2–3 minutes was decolorized with acid alcohol and followed by alkaline alcohol. This stain was used only on unsectioned material, and gave excellent results.

Field observations

About the middle of March, 1926, *Vaucheria geminata* made its first flourishing appearance after the winter season. The period of vigorous growth lasted until about the first of May. Sexual organs developed during the latter part of April and the early part of May.

About the same time that *Vaucheria* was observed in its natural habitats, oospores were successfully germinated in the laboratory from cultures that had been kept there from the previous autumn. Fig. 16 shows a camera lucida sketch of a germinating oospore in the living condition. Other germinating oospores were observed, but not in sufficient numbers to section, and for the most part were older than the stage shown in the figure. Only one stage was observed younger than the one shown.

From June until August scarcely more than an occasional fragment of a filament could be found. This might have been due in part to the excessive dryness of the soil prevailing during this period. In the earlier part of July a 3-day period of rain and fog occurred, however, and following this period no signs of *V. geminata* could be found. During the first week in August another period of rain and fog occurred, similar to that of July. Within a week filaments of

Vaucheria could be observed with a hand lens. The plant increased in abundance until it almost covered the ground by the latter part of September. Sexual organs began to appear about the middle of this month, and were found in abundance during the earlier part of October. The sexual organs seemed to be more numerous in the fall than in the spring. The plant was green and appeared to be in good condition until injured by frosts the latter part of October. After heavy freezing weather, until about the middle of March, only fragments of living filaments could be found. These were always in close contact with the soil, indicating that *V. geminata* winters over, in part at least, by means of these fragments which resist freezing.

Field observations in 1925 gave the same results. *V. geminata* has two periods of vigorous development, one in the spring, from March to May, the other in the late summer and early fall, from August to the time of heavy frost. In the second period of activity the plant is more vigorously developed than in the first.

Cytological observations

The oogonia and antheridia arise from a process which branches off the main filament. Both male and female organs are borne on the same stalk. The oogonia arise as swellings of the tips of lateral filaments, while the antheridium occupies a central position and continues as an elongation of the terminal portion of the original stalk, but somewhat more slender, curving downward between the oogonia. The antheridial cell is soon cut off from the remainder of the plant by a partition, the male elements developing in the cell thus formed. The oogonia increase rapidly in size, and at the same time show the presence of numerous vegetative nuclei. When the oogonium has attained considerable size, a mass of dense cytoplasm occurs in the apical portion, in which is located a single nucleus. This nucleus is much larger than the vegetative nuclei, and always occupies a position in the apical portion of the oogonium (fig. 2). The oogonium continues to increase in size, while the apical portion tends to curve downward. As the egg nucleus is being carried forward in the cytoplasmic mass large vacuoles arise between the center and the apex of the oogonium. The vegetative nuclei are left behind

the vacuolated area. They show no signs of migrating either to or from the oogonium.

One or two periods of general mitosis occur in the oogonium when nearly all the nuclei are in some phase of division. The egg nucleus, which has been set off from the vegetative nuclei in a dense mass of cytoplasm, shows mitotic stages in advance of those in the vegetative nuclei; that is, when the gamete nucleus is in anaphase the vegetative nuclei are in metaphase or earlier stages of mitosis. The gamete nucleus undergoes a mitosis which strongly indicates a reduction phenomenon. Figs. 7 and 7*a* show a gamete nucleus in the anaphase. Five distinct chromosomes are in one group while there are five in the other group less distinctly visible. Distinct metaphases were observed in the vegetative nuclei of the same oogonium (figs. 6, 6*a*, 6*b*). Ten chromosomes were observed in the metaphase of a vegetative nucleus (figs. 6, 6*a*); hence the grouping of the chromosomes into fives in the gamete nucleus probably indicates a reduction condition. Another vegetative nucleus in the same oogonium shows the chromosomes on the equatorial plate with distinct spindles and no nuclear membrane present. A centrosome is plainly visible at one of the poles, while it is very faint at the other (figs. 6, 6*b*).

Nuclei destined to become female gamete nuclei are shown in figs. 2, 2*a*, and 3. These nuclei, from young oogonia in which there is no trace of a cross wall, are certainly in an early prophase condition, as indicated by the large granules of chromatin scattered throughout the nucleus and by the absence of a karyosome. The vegetative nuclei, so far as could be determined, are all in a resting condition in the oogonia from which these drawings were made.

Figs. 3, 2*a*, 4*a*, and 7*a* represent a series of stages in the development of the egg nucleus. In fig. 3 an early prophase with about twenty distinct chromatin granules may be seen. At a later stage these granules are seen (fig. 2*a*) to be aggregated into less than ten larger masses. This is a stage in the organization of bivalent chromosomes, which is better seen in the anaphase shown in figs. 7 and 7*a*. After mitosis is complete it is seen that five chromatic bodies remain in the mature egg nucleus, while the remainder have passed into a small mass applied like a cap to the side of the egg nucleus

(fig. 4*a*). The whole process bears a strong resemblance to polar body formation.

The method of formation of the cross wall conforms to the description of DAVIS (2), except that it does not take place until after fertilization. The origin of the cross wall is also very similar to the same phenomenon described by HARPER in *Pilobolus* and *Sporodinia;* by SWINGLE (9) in *Rhizopus* and *Phycomyces;* and by YAMANOUCHI (12) in *Polysiphonia.* After the male element enters the oogonium, vacuoles begin to form in the region where the cross wall is to appear (fig. 13). These fuse until they form a partition across the stalk of the oogonium, and finally separate it from the parent filament. The cross wall is formed along the boundaries of the large flat vacuole, which now completely separates the oogonium and the filament.

There is no evidence in the writer's preparations in support of OLTMANNS' contention that there is a migration of nuclei from the oogonium before the formation of the cross wall. At the time of fertilization the vegetative nuclei are as abundant in the oogonium as at any other time. The membrane of the partition begins to form immediately after fertilization, and in all probability is completely formed by the time fusion of the male and female nuclei occurs. Evidence is in support of DAVIS' contention, that the nuclei begin to disintegrate about the time of the beginning of the formation of the cross partition. Before fusion of the gamete nuclei (fig. 14), resting nuclei are still present, although many seem to have lost their nuclear membrane in a manner comparable with the description of DAVIS. Fig. 15 shows a condition in which the gamete nuclei have fused, the fusion nucleus has migrated to the center of the oospore, and of the vegetative nuclei, only a trace of chromatin material here and there is visible. The cross wall has completely formed in this condition.

When fertilization occurs, the large vacuoles (which have been occupying a more or less centro-apical position) become smaller, and a structure (figs. 13, 14) which seems to have cytoplasmic connections with the dense cytoplasm about the gamete nuclei is formed. When the male nucleus has attained a size nearly equal to that of the female, the gamete nuclei fuse and the vacuolated structure

and dense cytoplasm disappear. Finally the zygote nucleus occupies a position in the center of the oospore.

A coenocentrum is not present in *V. geminata* unless the dense mass of cytoplasm in which the egg nucleus is located be homologized with such a structure. This is a mass of granular cytoplasm which differs from the rest of the cytoplasm by being more dense and not vacuolar. It is not a spherical structure, but has a more or less definite semiellipsoidal shape. In this the egg nucleus undergoes mitosis. This structure does not occur in the center of the oogonium, as has been described by WAGER (11) and others as characteristic of coenocytic plants. It is located near the apical portion of the oogonium just behind the "receptive spot." It is certainly a dynamic center of activity, as is shown by the behavior of the egg nucleus.

The writer's preparations show on the average a considerably greater number of vegetative nuclei in an oogonium than DAVIS (2) estimated. He stated that the range was from about 20 to 50; I find from several counts that the range in my material is from 55 to 141. It is probable that DAVIS' counts were made in young oogonia, before the number of vegetative nuclei was increased by mitotic division. DAVIS was of the opinion that the nuclei are carried into the young oogonium by the inflow of the cytoplasm; evidence from my material indicates that there is no nuclear migration to or from the oogonium in *V. geminata*.

Earlier investigators were of the opinion that all of the vegetative nuclei in an oogonium are potentially gamete nuclei. I am convinced that this does not hold true in the case of *V. geminata*. The gamete nucleus is set off from the vegetative nuclei early in the development of the oogonium. It occupies an apical position with respect to the oogonium, and is always seated in a dense granular mass of cytoplasm in which no vegetative nuclei are found. At the same time the vegetative nuclei occupy the two-thirds of the oogonium nearest the stalk. This indicates that the gamete nucleus is distinctly differentiated from the vegetative nuclei early in the development of the oogonium.

The problems of the time of differentiation of the gamete nucleus and of its relation to the vegetative nuclei are of considerable importance and difficulty. The oogonia arise as swellings of the tips

of lateral filaments borne on a stalk branching from the main filament, and are in a sense comparable with the tips of vegetative filaments. In actively growing points the vegetative nuclei are more abundant near the apical portion than farther back in the filament (fig. 17). At the same time many specimens show that the cytoplasm is more dense near the tip, and resembles in appearance the cytoplasm found in the "receptive region" of the oogonium. In this mass several nuclei are located.

The phenomenon of mitosis makes itself apparent in the oogonium of *V. geminata* when it has nearly reached its full size. The egg nucleus lies in a dense mass of cytoplasm just behind the receptive region. It is considerably larger than the vegetative nuclei, and no vegetative nuclei are found near it. Before mitosis is distinguishable in the vegetative nuclei, they all present a typical resting condition in having a large karyosome, more or less centrally located, and a clearly outlined nuclear membrane. The enlarged egg nucleus has the chromatin scattered throughout it in the form of numerous small granules (fig. 3). This condition is followed by a decrease in the number of granules present in these stages. A metaphase of the egg nucleus has not been observed. Fig. 7 shows a section of an oogonium containing the egg nucleus in a typical anaphase. Five chromosomes are in one group, while the other five are in a denser group and are less distinctly stained. Another section from this same oogonium (fig. 6) shows the vegetative nuclei in prophase and metaphase stages, and a few resting nuclei. Fig. 6*a* shows a vegetative nucleus in which ten chromosomes were counted. The enlarged drawing shows these chromosomes and their relation to each other as sketched with a camera lucida under considerably higher magnification. No spindle fibers were visible in this mitotic figure. Fig. 6*b*, however, under higher magnification, shows a vegetative nucleus with distinct spindle fibers and two centrosomes, one less distinct than the other. This situation shows that the egg nucleus undergoes mitosis, and that it is in a more advanced stage than any of the vegetative nuclei in the same oogonium. A still more advanced stage of mitosis in the egg nucleus, a late telophase, is shown in figs. 4 and 4*a*. The egg nucleus has its membrane, but on one side is a distinct swelling containing a mass of chromatin. The egg nucleus

proper shows several large chromatin granules scattered through it. The vegetative nuclei are in late anaphases and early telophases.

While these nuclear changes are taking place, the central portion of the oogonium is becoming filled with large vacuoles. Fig. 5 illustrates another section of the same oogonium as fig. 4, showing the vegetative nuclei in late stages of mitosis and a few in the resting condition. The vegetative nuclei are seen to be much more numerous here than in fig. 2. Whether there have been one or two periods of general mitosis between the stages represented in these figures is impossible to say; however, eighteen vegetative nuclei are present in the section of fig. 2, while there are about forty-five in fig. 5. When the mitosis is completed, in the section shown in fig. 5, there will be something like seventy-five or eighty vegetative nuclei present. This situation shows that if there were approximately the same number of vegetative nuclei present in the oogonium of fig. 5 as were present in the oogonium of fig. 2 when the two oogonia were in the same stage of development, then certainly two periods of general mitosis must have occurred in the oogonium of fig. 5 in order to account for the increase as indicated. The vacuolated structure of the oogonium and the presence of a large number of vegetative nuclei, many of which are in stages of mitosis, indicate that the development of the egg is complete or nearly so. Figs. 4, 4*a*, 5, 7, and 7*a* support this view.

The process of fertilization seems to be effected by the antheridium and oogonium coming in close contact. A swelling occurs upon the apical portion of the oogonium, probably due in part to the wall of the oogonium becoming thin in that region (figs. 8–10). This condition was observed in living as well as in fixed material. The structure resembles somewhat that described by STEVENS (8) for *Albugo bliti*, and (7) for *Albugo portulacae*, and termed the "receptive papilla."

The phenomenon of fertilization was observed only in material fixed at 2:30 A.M., while mitotic figures were found only in the egg nuclei of material fixed at 2:30 P.M. This would indicate that during the afternoon the vegetative nuclei and the egg nucleus undergo mitotic divisions; while fertilization, the formation of the cross wall, and the maturing of the zygote occur in the latter part of the night

and early morning. When the male nucleus enters the oogonium it advances toward the egg nucleus (fig. 11), but does not fuse with it immediately (figs. 14, 8, 8*a*); it lies close beside it, and there grows until it has reached approximately the size of the female gamete nucleus (fig. 14). Then fusion occurs, while the fusion nucleus passes toward the center of the oosphere (fig. 15).

Discussion

Vaucheria geminata shows periodic occurrence. Whether the controlling factor is length of day or temperature is a point of interest. The seasons of spring and autumn are without doubt most favorable for its development, both vegetatively and reproductively. Observations have been made on material growing under natural conditions. The same general conditions seem to exist in every case. *V. sessilis* was observed to be in a high state of vegetative development in a greenhouse in midwinter, while *V. geminata* a few feet away was barely living and presenting a yellowish green appearance, with only enough of the filaments present to make it apparent to the naked eye. Why was *V. geminata* not in a vigorous stage of development while *V. sessilis* was, under the same conditions? Similar observations were made during the summer months from June to August, and a condition was found comparable with that of the midwinter season.

Observations on photoperiodism by GARNER and ALLARD (3) indicate the possibility that *V. geminata* in its periodical development may be influenced by the length of the day. Comparing the time between sunrise and sunset of the two periods when reproduction is becoming fairly evident, we find that the length of the day in spring (April 15) is 13 hours, 13 minutes; in the fall (September 15) it is 12 hours, 28 minutes. Hence these observations indicate that a day of approximately 12.75 hours is the optimum one for vigorous development of *V. geminata*.

Anyone undertaking a study of this plant will encounter serious difficulty in obtaining material in the proper vegetative and reproductive stages to secure evidence on every point presented in this paper. In this work the principal material from which these studies

were made was collected about the middle of October, and treated as previously described. Many other collections were made at various times, but in no case were they so favorable for study. Probably this condition accounts for the difficulty experienced by earlier investigators in correctly interpreting the actual conditions that accompany sexual reproduction in *Vaucheria*. Previous investigators were more or less interested in some one point and overlooked or misinterpreted many others. For example, DAVIS (2) was chiefly concerned with disproving OLTMANNS' theory of the migration of the nuclei from the oogonium, and to secure some points of similarity between *Vaucheria* and certain of the fungi. Since DAVIS worked with the same species that I have worked with, *V. geminata*, it is evident that the material with which he worked lacked some of the critical stages, or that he overlooked them if they were present. The position of the egg nucleus, the absence of mitotic figures, and other points which he described concerning the oogonium are quite different from those found by the writer. OLTMANNS' theory of the migration of the nuclei from the oogonium before the formation of the cross partition has no support from my observations, even though his theory has been confirmed by a student of his. Possibly the situation is different in *V. sessilis*, but from the few preparations made of this material I find no evidence supporting his contention.

DAVIS's theory of the vegetative nuclei flowing into the oogonium with the inward flow of the cytoplasm gets no support from my observations. Strong evidence is obtained from the previous study that the progenitors of the gamete nuclei are located in the apical portion of the growing filament. All of the nuclei, both vegetative and reproductive, found in the oogonium arise as a result of mitotic divisions occurring within the oogonium. Early in the development of the oogonium the nucleus destined to become the gamete nucleus becomes surrounded by a dense mass of cytoplasm. As the oogonium grows, the egg nucleus is carried forward in this mass, leaving the vegetative nuclei behind. The vegetative nuclei occupy the proximal two-thirds of the oogonium, while the egg nucleus lies in the distal third. When the egg nucleus has completed the last maturation division, the vegetative nuclei have attained their greatest number

within the oogonium. The fact that many vegetative nuclei arise after the functional egg nucleus has become differentiated overthrows an old idea that all of the vegetative nuclei are potential gamete nuclei.

Summary

1. *Vaucheria geminata* is found most abundantly in the spring and autumn. Sexual reproductive organs are most abundant in September and October; they are also found about April 15.

2. There are one or two periods of general mitosis in the oogonium. The egg nucleus undergoes mitosis just in advance of the vegetative nuclei.

3. The nuclear membrane disappears in both the vegetative and reproductive nuclei during mitosis. Spindle fibers are visible in the vegetative nuclei in the metaphase.

4. The cross wall begins to form about the time the sperm enters the oogonium, and is of vacuolar origin. The vegetative nuclei begin disintegration at the time of fertilization.

5. There is no migration of nuclei either to or from the oogonium of *Vaucheria geminata*.

6. A swelling forms from the oogonium in the "receptive region," which comes in contact with the antheridium at the time of fertilization. The male element enters through this process.

7. Fertilization occurs during the latter part of the night. Maturation divisions in the egg nucleus occur in the afternoon. The formation of the cross wall, the disintegration of the vegetative nuclei, and the fusion of the gamete nuclei occur in the latter part of the night and early morning.

8. The number of vegetative nuclei in the oogonium varies in specimens examined from 55 to 141. They are increased considerably in number during the periods of mitosis.

9. The fusion of the gamete nuclei does not occur immediately when the male element enters the oogonium. The male nucleus lies beside the egg nucleus and there enlarges to approximately the size of the female gamete nucleus.

10. In the center of the oogonium a vacuolar structure forms after fertilization. The zygote nucleus takes its position in the midst of this structure.

Professor I. F. Lewis deserves special mention in connection with this work, for it was through his advice and suggestions, together with his close observation of preparations presented to him, that this work was made possible.

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EXPLANATION OF PLATES XIII, XIV

PLATE XIII

FIGS. 1, 2, 4, 5, 6, 7 ×725; figs. 2*a*, 3, 4*a*, 6*a*, 6*b*, 7*a* ×1850.

FIG. 1.—Section of young oogonium.

FIG. 2.—Oogonium showing large female nucleus; no signs of a cross wall.

FIG. 2*a*.—Female nucleus of fig. 2, showing structure under higher magnification.

FIG. 3.—Early prophase condition of female nucleus.

FIG. 4.—Vegetative and reproductive nuclei in states of mitosis.

FIG. 4*a*.—Female nucleus of fig. 4 highly magnified.

FIG. 5.—Section from same oogonium as fig. 4, showing vegetative nuclei and their relation to stalk.

FIG. 6.—Section of oogonium showing vegetative nuclei in stages of mitosis: *a*, ten chromosomes in metaphase stage; *b*, distinct spindle fibers.

FIGS. 6*a*, 6*b*.—Vegetative nuclei highly magnified.

FIG. 7.—Female nucleus showing two groups of five chromosomes each.

FIG. 7*a*.—Female nucleus of fig. 7 highly magnified.

PLATE XIV

FIGS. 8, 9 ×425; fig. 10 ×475; figs. 8*a*, 12, 15 ×1850; figs. 11, 13, 14, 15 ×725; fig. 16 ×50.

FIGS. 8–10.—Sections from same oogonium; note position of antheridium, also vacuoles in center and in region of cross partition of oogonium.

FIG. 8*a*.—Gamete nucleus of fig. 8 highly magnified.

FIG. 11.—Receptive region of oogonium with egg nucleus and approaching sperm.

FIG. 12.—Nuclei of fig. 11 highly magnified.

FIG. 13.—Oogonium with male and female nuclei in close contact, showing numerous vacuoles in region where cross wall will form; note disintegrating condition of vegetative nuclei with the loss of membranes.

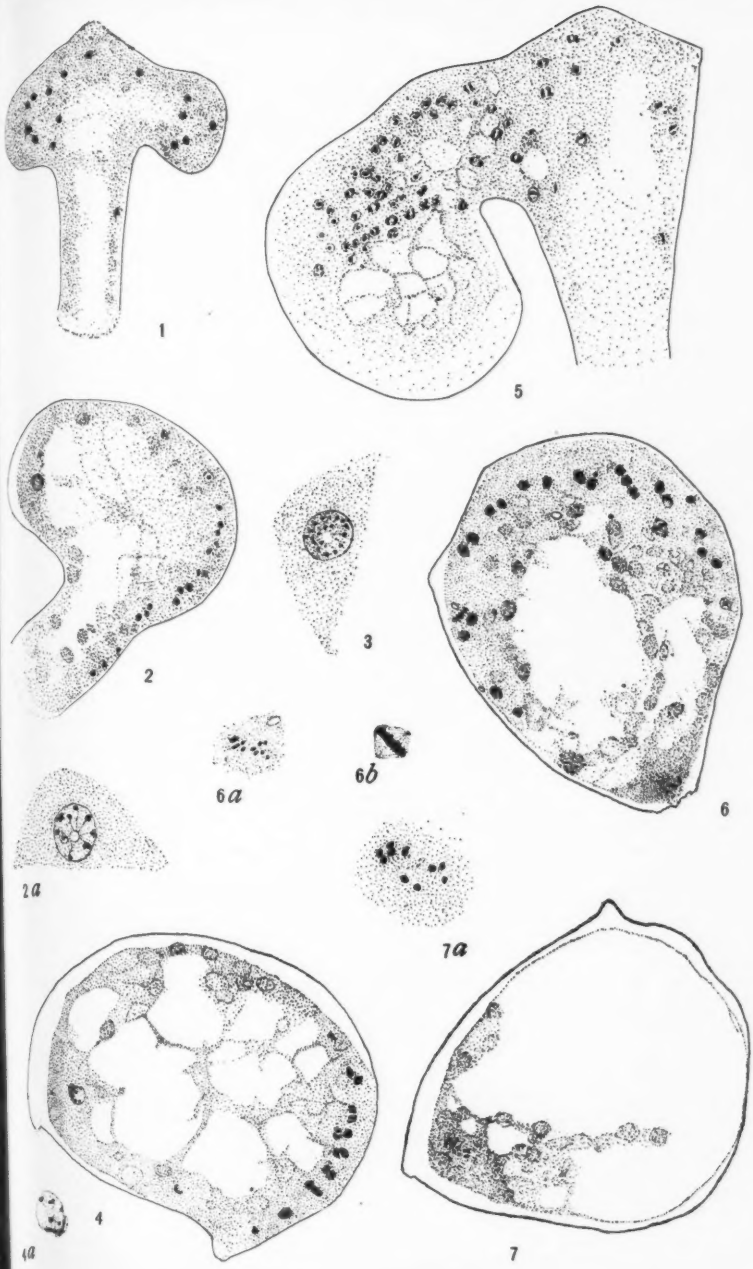
FIG. 14.—From an *in toto* preparation stained with Delafield's haematoxylin; male and female gamete nuclei approximately same size; vacuoles numerous in center of oogonium; vegetative nuclei in stages of disintegration.

FIG. 15.—Mature zygote at center of oosphere; cross wall completely formed; fragments of vegetative nuclei observable.

FIG. 15*a*.—Zygote of fig. 15 more highly magnified; note structure of nucleus.

FIG. 16.—Germinating oogonium with old hull still clinging to base: *a*, *a'*, filaments; *b*, *b'*, rhizoids.

FIG. 17.—Tip of young vegetative filament; note numerous nuclei near growing tip.



MUNDIE on VAUCHERIA

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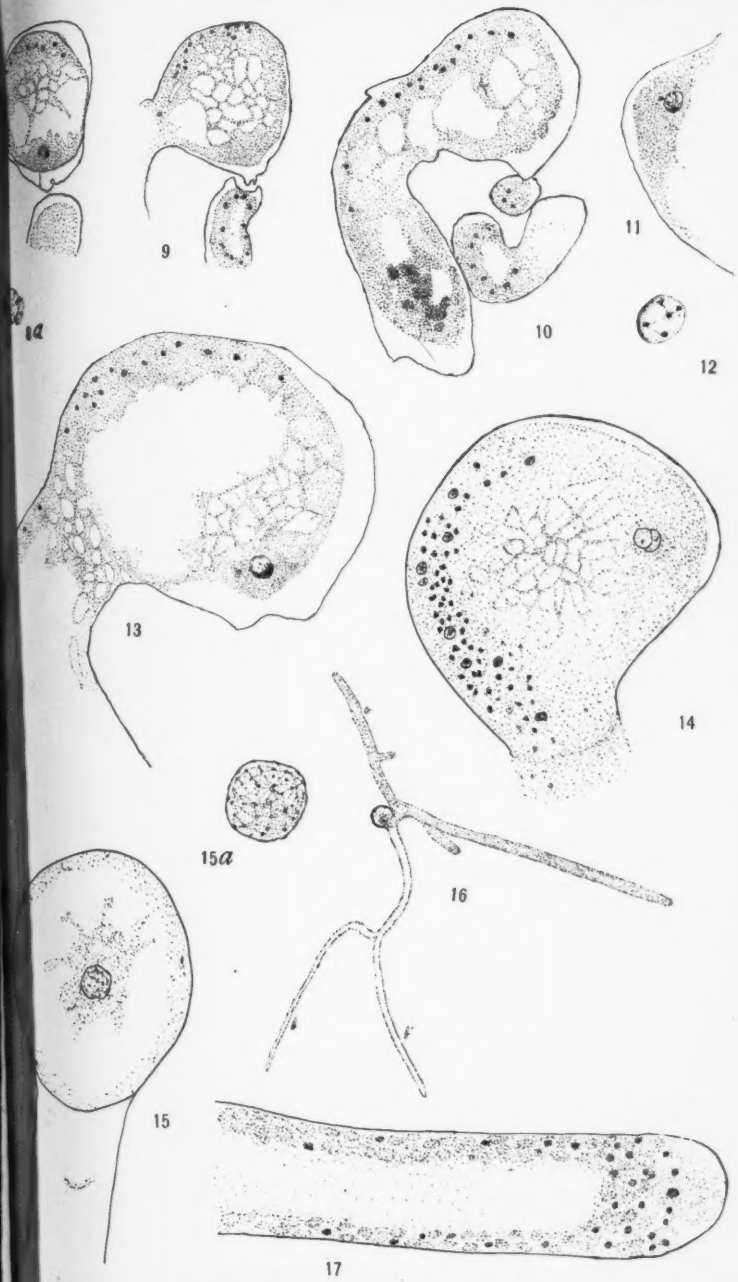

MUNDIE on VAUCHERIA

DEVELOPMENTAL HISTORY OF THE FRUIT IN LINES OF CUCURBITA PEPO DIFFERING IN FRUIT SHAPE

EDMUND W. SINNOTT AND GEORGE B. DURHAM

(WITH ONE FIGURE)

Introduction

Investigations on the heredity of form of leaves, fruits, and other organs in various species of plants show in most instances that characters of this sort follow the same method of inheritance as do other quantitative and qualitative traits. The problem is somewhat complicated here, however, by the fact that what is inherited is not a simple character which may be measured directly, but consists in a ratio between two or more dimensions, and thus involves the general problem of growth correlations and their inheritance. Careful quantitative studies of heredity in such traits have not been numerous, but are greatly to be desired in view of the light which they may throw on developmental problems.

The senior author [1, 2] has for some time been studying the inheritance of fruit shape in *Cucurbita pepo*, as measured by dimensional indices for the fruit as a whole and for its various parts, and has attempted to analyze his results in Mendelian terms. On the assumption of the operation of genetic factors which control various degrees of flattening or elongation, and which display the familiar phenomena of dominance, segregation, independent assortment, linkage, and cumulative and inhibitory effects, it has been found possible to explain the results of fruit shape inheritance in this species in a somewhat satisfactory manner. Studies of the same sort by LEAKE, KEARNEY, LINDSTROM, and others have led to similar conclusions for their material. Particular attention was paid to the earlier developmental stages.

[1] SINNOTT, E. W., Inheritance of fruit shape in *Cucurbita pepo*. BOT. GAZ. 74:95–105. 1922.

[2] ——, A factorial analysis of certain shape characters in squash fruits. Amer. Nat. 61:333–344. 1927.

A genetic analysis of such traits no longer satisfies students of heredity, however, who are increasingly concerned with the developmental phases of their problems, and are endeavoring to understand the mechanism by which a particular gene controls the development of a particular character. A necessary preliminary to such a study, particularly in quantitative traits, is a knowledge of the

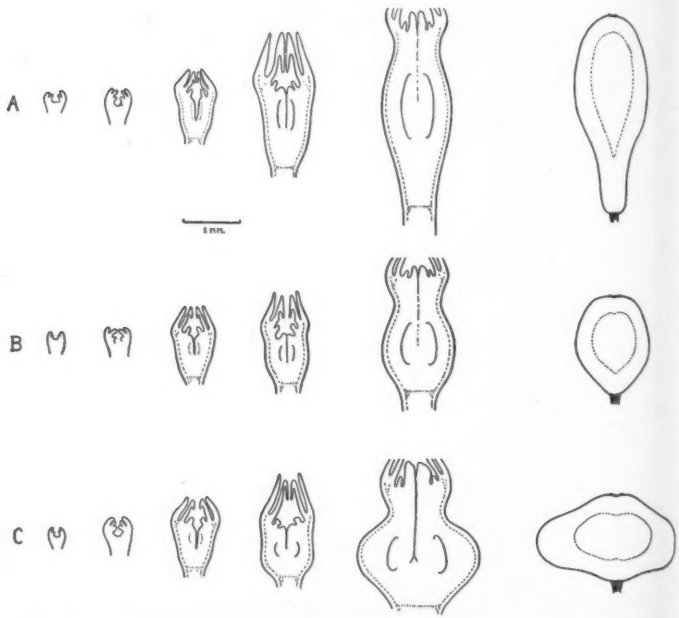

FIG. 1.—Outlines, from camera lucida drawings, of longitudinal sections through successive stages in development of ovary and fruit in elongate type (*A*), spherical type (*B*), and disk type (*C*) of squash fruits. First five stages drawn to scale. First shows early floral primordium and rudiments of sepals and petals; second shows beginning of carpellary cavity and rudiments of staminodia; third shows elongation of ovarian region and beginning of carpellary differentiation; fourth shows outline of central carpellary tissue clearly marked and (in disk type) commencement of equatorial thickening of wall; fifth shows young ovary in essentially its final shape. Dotted lines represent course of vascular bundles. From the fourth stage shown here, the three shape types begin to be clearly distinguishable. Outlines at extreme right show mature fruit shape which develops from each of these primordial series.

stages, from inception to maturity, which are traversed by the structure displaying the traits in question. It is important, for example, to measure the shape of an organ in its various developmental stages, and to determine from microscopical observation those differences in the extent and localization of growth which are responsible for the shape ultimately produced. Particularly favorable material for a study of this sort is provided by our squash lines, and the present paper sets forth the results of an investigation of the development of the fruit, from floral primordium to maturity, in pure lines of *Cucurbita pepo* which differ markedly in fruit shape, and in which the differences have been subjected to genetic analysis. The specific effect upon development of various genotypes for shape can in this way be traced.

Plants were used from fifteen lines which have been inbred for from six to thirteen years, and which now breed very true for all their characters. These include three main shape types: the spherical, in which the length and width of the fruit are essentially equal at maturity; the elongate, in which the length is much greater than the width; and the disk, in which the width is much greater than the length. These are shown in outline in the right-hand column of fig. 1. Intermediates of all sorts may occur. The methods used in determining and plotting shape indices have been described in a previous paper (see footnote 2).

Stem tips from which most of the leaf primordia had been removed were killed in formol-acetic-alcohol, imbedded in paraffin, and sectioned for study of the earliest stages in ovary development. After the ovary primordia had reached a length of a few millimeters and could be handled separately, they were removed individually and killed and imbedded. Sections, both longitudinal and transverse, were made of all the stages in all the important lines. For late ovary and for fruit stages, macroscopic studies were made of the entire structure, and tissues from various portions were examined microscopically.

Results

The morphology and development of the pistillate flower of the Cucurbitaceae have been studied by many botanists, and a general survey of this subject for the family as a whole is well presented by

KIRKWOOD.[3] Results from the material studied by us were found to conform closely to those obtained by previous workers, and are outlined diagrammatically in fig. 1, where the earliest stages are shown at the left and the progressively older ones toward the right. The rudiment of a pistillate flower first appears as a rounded swelling just below the growing tip of the stem. This swelling soon becomes broadened and flattened at its tip, and from the edge of the platform thus produced arise five prominences, the sepal primordia. Inside these and alternating with them soon appear five others, the petal primordia; and following these a circle of staminodia, which soon cease growth and are inconspicuous at anthesis. Meanwhile the tissue at the edge of the platform from which these circles spring elongates somewhat, producing a shallow concavity. At each of three points (sometimes four or five) on the inner wall of this cavity appears a ridge, and these ridges grow inward toward the center of the cavity, where they meet. These are the carpellary ridges, and along their recurved edges, from the base of the cavity to a point just below the level of the perianth primordia, develop the complex placentae where the ovules are ultimately borne. The ridges extend above this level and later fuse to form the style.

The morphology of the inferior ovary which is thus produced has been a matter of debate. Some botanists hold that the carpels give rise only to the stigmas, style, and upper portions of the ovary, the lower region being formed entirely from the floral axis. Others believe that the carpels constitute the entire ovuliferous tissue, lying with their backs to the ovary wall and bearing the ovules on their inrolled margins. The ovary wall itself is thought by some to be a downward prolongation of the perianth circles, but by others is regarded as an upward extension of a hollowed receptacle, to the inner face of which the carpels have become fused. The latter view is probably the most widely accepted today, and, at least for purposes of description, is adopted here. Certainly in many cases a rather definite line is visible, in early stages of the ovary, between an inner tissue, clearly continuous with the carpellary folds and consisting chiefly of cells elongated longitudinally, and an outer layer

[3] KIRKWOOD, J. E., The comparative embryology of the Cucurbitaceae. Bull. New York Bot. Gard. 3:313–402. 1904.

in which most of the cells are elongated in a tangential direction. At a very early stage, when the first perianth primordia are developing, the procambial strands for ten vascular bundles appear, running up from the peduncle into the floral rudiment. These later branch repeatedly, but the ring of bundles thus formed remains relatively close to the outer surface of the ovary. These strands arise from a plexus of bundles at the base of the flower, where it joins the peduncle. Several definite layers may thus be recognized in the wall of the ovary and fruit: (1) the epidermis; (2) the cortical layer of the receptacle, relatively narrow; (3) the ring of vascular bundles; (4) the medullary layer of the receptacle, relatively wide; (5) the sterile outer layer of carpellary tissue; and (6) the central ovuliferous carpellary mass.

In developing ovaries of all the types studied, all these component layers are present, but the ovaries themselves differ markedly in shape. Indeed, the most striking fact which a comparative study of the various types discloses is that from almost the very beginning of differentiation each shape type is clearly distinguishable. In the gross differences in relative length and width of the ovary which determine these shape differences, however, the specific tissue layers do not share equally, their development relative to one another varying from type to type. Areas of localized growth also play an important part in the ultimate shape produced. Growth in such an organ as this is not a generalized phenomenon, therefore, affecting the organ uniformly and as a whole; but is a much more complex affair, and seems to be governed, at least to a certain extent, by factors which control particular processes or particular regions. The importance of this concept in problems of morphogenesis, particularly those concerned with the effect of genetic factors upon growth and form, is obvious.

A description of the developmental history of the more important shape types in squash fruits well illustrates this phenomenon of differential growth.

In all the shape types the very earliest stages are similar, but from a point soon after the floral primordium becomes flattened and begins to produce rudiments of the floral circles around its margin, differences begin to appear. Elongation of the tissue immediately

KIRKWOOD.[3] Results from the material studied by us were found to conform closely to those obtained by previous workers, and are outlined diagrammatically in fig. 1, where the earliest stages are shown at the left and the progressively older ones toward the right. The rudiment of a pistillate flower first appears as a rounded swelling just below the growing tip of the stem. This swelling soon becomes broadened and flattened at its tip, and from the edge of the platform thus produced arise five prominences, the sepal primordia. Inside these and alternating with them soon appear five others, the petal primordia; and following these a circle of staminodia, which soon cease growth and are inconspicuous at anthesis. Meanwhile the tissue at the edge of the platform from which these circles spring elongates somewhat, producing a shallow concavity. At each of three points (sometimes four or five) on the inner wall of this cavity appears a ridge, and these ridges grow inward toward the center of the cavity, where they meet. These are the carpellary ridges, and along their recurved edges, from the base of the cavity to a point just below the level of the perianth primordia, develop the complex placentae where the ovules are ultimately borne. The ridges extend above this level and later fuse to form the style.

The morphology of the inferior ovary which is thus produced has been a matter of debate. Some botanists hold that the carpels give rise only to the stigmas, style, and upper portions of the ovary, the lower region being formed entirely from the floral axis. Others believe that the carpels constitute the entire ovuliferous tissue, lying with their backs to the ovary wall and bearing the ovules on their inrolled margins. The ovary wall itself is thought by some to be a downward prolongation of the perianth circles, but by others is regarded as an upward extension of a hollowed receptacle, to the inner face of which the carpels have become fused. The latter view is probably the most widely accepted today, and, at least for purposes of description, is adopted here. Certainly in many cases a rather definite line is visible, in early stages of the ovary, between an inner tissue, clearly continuous with the carpellary folds and consisting chiefly of cells elongated longitudinally, and an outer layer

[3] KIRKWOOD, J. E., The comparative embryology of the Cucurbitaceae. Bull. New York Bot. Gard. 3:313–402. 1904.

in which most of the cells are elongated in a tangential direction. At a very early stage, when the first perianth primordia are developing, the procambial strands for ten vascular bundles appear, running up from the peduncle into the floral rudiment. These later branch repeatedly, but the ring of bundles thus formed remains relatively close to the outer surface of the ovary. These strands arise from a plexus of bundles at the base of the flower, where it joins the peduncle. Several definite layers may thus be recognized in the wall of the ovary and fruit: (1) the epidermis; (2) the cortical layer of the receptacle, relatively narrow; (3) the ring of vascular bundles; (4) the medullary layer of the receptacle, relatively wide; (5) the sterile outer layer of carpellary tissue; and (6) the central ovuliferous carpellary mass.

In developing ovaries of all the types studied, all these component layers are present, but the ovaries themselves differ markedly in shape. Indeed, the most striking fact which a comparative study of the various types discloses is that from almost the very beginning of differentiation each shape type is clearly distinguishable. In the gross differences in relative length and width of the ovary which determine these shape differences, however, the specific tissue layers do not share equally, their development relative to one another varying from type to type. Areas of localized growth also play an important part in the ultimate shape produced. Growth in such an organ as this is not a generalized phenomenon, therefore, affecting the organ uniformly and as a whole; but is a much more complex affair, and seems to be governed, at least to a certain extent, by factors which control particular processes or particular regions. The importance of this concept in problems of morphogenesis, particularly those concerned with the effect of genetic factors upon growth and form, is obvious.

A description of the developmental history of the more important shape types in squash fruits well illustrates this phenomenon of differential growth.

In all the shape types the very earliest stages are similar, but from a point soon after the floral primordium becomes flattened and begins to produce rudiments of the floral circles around its margin, differences begin to appear. Elongation of the tissue immediately

below the perianth rudiments, which carries them up above the floor of the cavity, proceeds at relatively different rates, in comparison with the growth in width, in the different types. In forms which will produce essentially spherical ovaries and fruits, this elongation is not markedly greater at first than the growth of the whole primordium in width, so that the structure from the tip of the peduncle to the base of the perianth rudiments is, throughout these early stages, not very much longer than wide. In the elongate types, however, growth in the longitudinal dimension is relatively much more rapid and lateral growth less so, so that from a very early stage the ovary primordium is much longer than wide. As development proceeds, growth in width keeps pace, proportionally, with growth in length, so that the ratio between these dimensions remains essentially constant.

It is noteworthy, however, that between these two types (spherical and elongate) there is almost no difference, at corresponding stages, in the width of the ovary wall (receptacular cortex, vascular cylinder, and pith), but that almost from the first the essential shape difference is one which concerns only the central carpellary tissue, which will ultimately develop into the seed cavity. Factors for shape in these types produce their effect by controlling the dimensions of this region, which, whatever its shape, is surrounded by a wall of uniform width.

Aside from this difference in the proportions of the ovarian cavity, the elongate types differ in another important particular from the spherical ones, namely, in the production of a sterile "neck" at the base of the ovary, between the cavity and the flower stalk (fig. 1 *A*). This begins its development after the elongation of the carpellary zone has started but while the whole primordium is still in a very early stage and is only a few millimeters long. The elongation of the neck carries up the rest of the primordium for some distance, but after an early period of rapid growth the ratio of neck length to the length of the rest of the ovary remains essentially constant. Growth in length of the neck is carried on for a time through the continued meristematic activity of the region at its base, nearest the peduncle, although in later stages growth is chiefly interstitial. The neck is clearly a part of the ovary and not a

continuation of the stalk, for the tissues of these two organs are readily distinguishable from one another and there is also a definite plexus of vascular bundles which separates them. The sterile outer layer of carpellary tissue often runs down for a considerable distance into the neck, forming a V-shaped area which can well be seen in longitudinal sections of mature fruits of this type.

The relative length of neck varies considerably in different lines. In disks and true spheres it is absent, in elongate spheres or fruits of the "Fordhook" type it is short, and in true elongates it may even exceed the ovarian cavity in length. Its development seems to be correlated to some extent with the shape of the ovary proper, but at least in certain cases it may be inherited independently of ovary shape, as is shown by the occurrence of "jug" fruits in the descendants of crosses between disks and elongates. Here the ovarian part of the fruit is broad and flat, but is provided with a definite neck or "handle" at the base.

The disk or flat type of ovary and fruit differs from the sphere and the elongate partly by the shape of the ovarian cavity or central carpellary tissue, which is relatively much shorter and broader in these forms. This manifests itself early in the development of the primordium by the greater width of the platform and the comparatively small amount of elongation. In one of our lines of disks, grown from a Mandan Indian race of squashes, this flattening of the ovarian cavity is the only respect in which the ovary and fruit differ from the more elongate types, for in this race the ovary wall (and later the pericarp) is of essentially uniform thickness at all points, and the ovary thus resembles that which would be produced by the marked flattening of a spherical type.

The other disk lines studied, however, show not only this flattening of the ovarian cavity, but, in addition, a marked increase in the relative thickness of the ovary wall, particularly in the equatorial region, which gives rise to the scallop or border so characteristic of the fruit of these types. This thickening does not affect all the wall layers, but is confined to the inner one consisting of the receptacular pith and the outer carpellary zone, the distinction between which is here difficult to observe. It does not affect the cortex (that portion outside the vascular ring), which is no wider here than in corre-

sponding stages of spherical and elongate types. This localized lateral growth makes its appearance at an early period in the development of the flower, about the time that the ovarian cavity becomes closed. It is initiated by numerous and somewhat regular tangential divisions, which soon expand the ovary wall in the equatorial region to a thickness considerably greater than that above and below; and this relative thickness, thus early attained, persists throughout the development of the flower and fruit. The position and the degree of development of this zone vary considerably in different races. In some it is strongly marked, producing a much flattened fruit type, but in others it is far less conspicuous. It may be considerably nearer one pole than the other, producing in consequence a disk with the appearance of either a reflexed or an upturned border. It is usually more strongly developed at certain points than at others, producing marked teeth or ribs. This equatorial thickening is sometimes found in squashes in which the ovarian cavity is clearly of the spherical or the elongate type, but here it is rarely as well developed or sharply localized as in disk forms. Undoubtedly, however, as in the case of the neck, the development of this tissue is a trait which may be inherited, in part, quite independently of the character of the rest of the ovary.

Each shape type is distinguishable from a very early stage, certainly from a period when the ovary is no more than 1 mm. in width. The shape of the ovary during its early development is somewhat more elongate than that which it assumes in the flower and fruit, doubtless because of its more pronounced growth in width at the time when the ovules mature. The remarkable fact, however, is that in its major aspects fruit shape is established at an extremely early period of development, when the volume of the ovary primordium is often less than one-millionth of the volume of the fruit at maturity.

No difference in size or shape of cells could be observed between the various squash types studied. In every case the cells of the early primordium are small and uniform in size. As development and differentiation progress, the cells of certain tissues, such as the pith of the receptacle, increase in size more rapidly than others, and as the ovary grows larger its component cells tend gradually to become

somewhat larger; although this increase is by no means proportional to the enlargement of the organ as a whole, which is chiefly effected by increase in cell number rather than by cell size. In the ripening fruit the increase in cell size is more conspicuous than in the early stages. In all cells, the actual volume of the nucleus changes relatively little during ovary development. It is noteworthy that the differences in relative dimensions observed in the various shape types are not correlated with differences in the relative dimensions of the cells composing them.

Discussion

Such a developmental study as this tends to emphasize certain general conclusions as to the determination of form. First, the form of the organ is established at a very early stage in its development, and as growth proceeds this shape is maintained, the relation of the various dimensions to one another remaining essentially constant (at least under similar environments). This must be due to a factor or factors controlling correlation of growth. Shape obviously cannot be merely a resultant of the extent of growth in a single dimension (such as length) in proportion to the total increase in bulk of the organ, since the ratio of a single dimension to total volume changes profoundly with increase in size, whereas shape does not change. Through some mechanism, be it the distribution of hormones or specific organ-forming substances, or the establishment of a gradient of some sort in a specific protoplasmic base, growth in one dimension or region is definitely correlated with growth elsewhere. Whatever the mechanism by which these shape differences are brought about, we can say that, in the present case at least, it involves control over two quite different processes or aspects of growth: (1) the plane of cell division in growing tissues, and (2) the localization of growth in various parts of the developing organ.

The shape differences in the ovary proper, in this species, result from the fact that growth in one dimension (length) is relatively more rapid or less rapid than growth in the other dimension (width). Since the cells themselves are of essentially the same size and shape in the various shape types, these differences are evidently due to more frequent cell divisions in one plane than in the other. What-

ever it is that controls the polarity of the cells, and thus determines the orientation of the equatorial plate at mitosis, determines to a very considerable extent the organic form which is ultimately produced. Cell polarity may be said to be one of the fundamental problems of morphology.

The second aspect of growth control is also of importance, because growth is not uniformly distributed through the tissues of a developing organ, but is often somewhat localized and proceeds at different rates in different regions, with a marked effect on the form produced. The fact that in certain lines of squashes an active growing region between the base of the ovary and the cavity is responsible for the development of a conspicuous neck in these types, and the rapid growth of the inner ovary wall in the equatorial region of most disk types (which produces the border so characteristic of them) are examples of such localized growth.

The shapes resulting from growth thus controlled and localized are constant within lines which are genetically pure, and breeding investigations have shown that they are transmitted in inheritance in a manner essentially Mendelian and similar to that shown by other characters of the plant. It is therefore fair to conclude that the specific differences in growth correlation which result in these shape differences are controlled by specific genes.

Summary

1. The inheritance of fruit shape in *Cucurbita pepo* may be ascribed to the operation of Mendelian factors.

2. In inbred and genetically pure lines showing the three main shapes of fruit (elongate, spherical, and disk), comparative studies were made of the developmental history of the various shape types from the earliest floral primordium to maturity.

3. From a very early stage in differentiation, when the ovary primordium is not more than one-millionth of the volume of the mature fruit, each of the various shape types is clearly distinguishable.

4. Much of the difference between the types, especially between the elongate and the spherical, was found to be due to shape differences involving only the central carpellary tissue (later the seed

cavity) and not the wall of the ovary and fruit, the latter maintaining an essentially constant width in fruits of diverse shapes.

5. In most of the disk types, however, not only is the seed cavity relatively short and broad, but there is also a localized thickening of the wall in the equatorial zone (involving only the inner wall layers) which results in a still flatter shape for fruits of this type.

6. In certain lines a sterile and relatively slender neck is developed at the base of the ovary.

7. The various fruit shapes are essentially similar in the size and shape of their component cells.

8. The factors determining shape are evidently those which govern growth correlation. They operate by controlling: (1) cell polarity, and thus the plane of cell division; and (2) the localization of growth in particular regions.

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COMPOSITION OF AVOCADO TREES IN RELATION TO CHLOROSIS AND TIP-BURN[1]

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The sensitivity of avocado trees to low temperatures has made it necessary that the trees be planted on slopes within short distances from the ocean, where suitable temperatures prevail. Such a restriction upon plantings, while eliminating the frost hazard, in many cases has not given suitable soil conditions for the growth of the trees. On the slopes extremely shallow soils are often encountered at the higher elevations, and frequently such hill sides show extensive outcroppings of limestone. In some coastal districts the water available for irrigation purposes contains considerable amounts of salts, such as chlorides and sulphates. The trees in such districts are subject to strong ocean winds, which although laden with moisture are an unknown factor in the desiccation of the leaves. HAAS and REED (2) have pointed out that dry winds may cause a temporary accumulation of salts within citrus leaves as a result of excessive transpiration, but the effect is not known as yet in regard to moist winds of high velocity. Chlorination of the irrigation water reservoirs in coastal districts is a common practice, and nothing is known in regard to the effect of such additions of chlorine upon the chlorine content of the irrigation water as it is delivered to the grower.

Heretofore the burning of avocado leaves has been ascribed to too long an interval between irrigations. This conclusion has been due to the fact that this tree is usually shallow rooted, and does not do well under extremely dry atmospheric conditions or under intense heat, but thrives best in the more humid coastal districts. Notwithstanding an adequate irrigation program, the leaves of avocado trees in many districts show tip-burn during late summer or early autumn, and progressive marginal burning[2] during the winter. Many if not all of these burned leaves fall the following spring as the new growth

[1] Paper no. 192, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

[2] The term tip-burn as commonly used by growers may indicate any stage of leaf burn that originates at the leaf tip.

appears, with the result that the sun's rays have full access to the branches and trunk. Young shoots may be killed as a result of sunburn, while the bark of older branches or the trunk may become dry and corky.

Very little is known in regard to the nutrition of avocado trees; in fact, some growers fertilize their trees very little, if at all, largely because of the difficulty of noting any relationship between fruiting and soil fertilization. The practices employed at present, in view of the lack of scientific data, are based largely on the results obtained in the culture of citrus. The present knowledge regarding cultural practices, and the frost resistance of the different varieties of avocado, has been well summarized by RYERSON, JAFFA, and GOSS (4). The present investigation is merely a beginning in the direction of an understanding of some of the difficulties encountered in avocado culture.

Many of the contoured slopes in the coastal districts show conspicuous outcroppings of limestone, so that the newly leveled soil above these outcroppings is frequently shallow and heavily charged with limestone. Newly planted trees in such locations may become chlorotic during the first season of growth or at any subsequent time. Applications of iron sulphate to the soil or injections of iron sulphate solution into the trees were found by THOMAS and HAAS (5) to be unsuccessful in overcoming the chlorosis, even temporarily, without injuring the trees. In such locations the generally accepted theory is that the trees are suffering from a faulty distribution of the iron supply within the tree. The function of iron is understood to be that of catalyzing the formation of chlorophyll, of which magnesium is a constituent. In chlorotic avocado leaves a reduced amount of chlorophyll is not necessarily accompanied by a reduction in the magnesium content of the leaves, as seen in table I. In such cases, however, the absorption of calcium by the leaves is excessive, so that the calcium present in relation to the magnesium is much greater than that occurring in normal leaves. The results obtained in table I for the calcium and magnesium content of chlorotic leaves do not necessarily mean that a definite ratio exists between calcium and magnesium in normal leaves, but rather that within a wide range of ratios the leaves may be expected to be normal.

In table II are given water-solubility data for the various inorganic constituents of dry avocado leaves. The ash of the soluble fraction represents relatively only a small part of the inorganic constituents of the dry matter. In the case of the chlorotic leaves the amount of ash of the water-insoluble portion is considerably larger than in the case of normal leaves. This is due to the large amount of insoluble calcium present. It is of interest to note the extremely low

TABLE I

EFFECT OF CHLOROSIS ON CALCIUM AND MAGNESIUM CONTENT OF AVOCADO LEAVES

VARIETY	LOCALITY IN CALIFORNIA	CONDITION OF LEAVES	ASH AS PERCENTAGE OF DRY MATTER	PERCENTAGE ASH		PERCENTAGE DRY MATTER		Ca/Mg
				Ca	Mg	Ca	Mg	
Fuerte.....	La Habra	Normal	9.60	16.64	11.52	1.597	1.106	1.444
Fuerte.....	Montebello	Leaf-burn	9.52	19.76	11.41	1.881	1.085	1.733
Puebla.....	La Habra	Normal	9.61	18.49	10.37	1.778	0.997	1.783
Fuerte.....	La Habra	Normal	10.33	15.84	10.32	1.638	1.067	1.535
Fuerte.....	Montebello	Leaf-burn	9.23	18.18	9.86	1.679	0.910	1.845
Puebla.....	La Habra	Normal	9.53	18.25	9.54	1.739	0.909	1.913
Fuerte.....	Brentwood	Normal	8.36	15.56	8.37	1.300	0.699	1.859
Fuerte.....	Culver City	Leaf-burn	9.64	19.24	8.03	1.855	0.775	2.394
Seedling....	Brentwood	Leaf-burn	9.48	17.43	7.70	1.653	0.730	2.264
Fuerte.....	La Habra	Chlorotic	9.04	16.78	5.90	1.517	0.534	2.841
Puebla.....	La Habra	Chlorotic	15.25	22.93	5.90	3.498	0.900	3.887
Fuerte.....	La Habra	Chlorotic	12.98	22.48	5.83	2.917	0.757	3.853
Lyon.....	La Habra	Chlorotic	8.36	19.20	5.36	1.606	0.449	3.577
Lyon.....	La Habra	Chlorotic	11.41	16.10	4.99	1.837	0.569	3.229
Fuerte.....	La Habra	Chlorotic	10.32	20.80	4.94	2.147	0.510	4.210
Puebla.....	La Habra	Chlorotic	18.63	25.97	4.77	4.840	0.889	5.444

water solubility in the case of the calcium of avocado leaves, as compared with the values given by HAAS (1) for citrus leaves. The calcium present in normal avocado leaves is considerably less than that present in citrus leaves. The magnesium content of avocado leaves, however, is from two to three times as great as that of citrus leaves. This large magnesium content may be of significance from the standpoint of the concentration of chlorophyll in avocado leaves and their capacity to manufacture carbohydrates, fats, and proteins. Practically all of the potassium is found in the water-soluble fraction. It is of interest to note the large concentration of potassium in the water-soluble fraction of the chlorotic leaves.

Table III shows the total phosphorus content of the dry matter

TABLE II

WATER-SOLUBILITY OF DRY MATTER OF MATURE AVOCADO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

Variety	Locality in California	Condition of leaves	Ash of		Calcium			Magnesium			Potassium			Sodium		
			Soluble fraction	Insoluble fraction	Soluble	Insoluble	Soluble as percentage of total	Soluble	Insoluble	Soluble as percentage of total	Soluble	Insoluble	Soluble as percentage of total	Soluble	Insoluble	Soluble as percentage of total
Fuerte	La Habra	Normal	3.52	6.81	0.086	1.552	5.25	0.747	0.320	70.01	0.508	0.026	95.13	0.104	0.061	63.03
Puebla	La Habra	Normal	3.05	6.49	0.097	1.642	5.58	0.587	0.322	64.57	0.678	0.034	95.22	0.135	0.090	60.00
Puebla	La Habra	Chlorotic	4.24	14.45	0.099	4.755	2.04	0.605	0.375	61.73	1.544	0.112	93.24	0.312	0.077	80.21

of the leaves as determined by the magnesium nitrate method. The values for the most part are not very different from those found for citrus leaves.

Table IV gives the percentage of total nitrogen in the dry matter of the leaves of four varieties of avocado. In some cases the nitrogen content is equal to that found in citrus leaves. When an avocado leaf burns, it is not known what becomes of the nitrogen in the burned area, whether it remains in the burned portion, or passes back into the unburned portion and remains there, or passes back into the shoots for new distribution. However, any water-soluble nitrogen remaining in the burned portion is likely to be leached out,

TABLE III

PHOSPHORUS CONTENT OF AVOCADO LEAVES

VARIETY	LOCALITY IN CALIFORNIA	CONDITION OF LEAVES	TOTAL PHOSPHORUS AS PERCENTAGE DRY MATTER
Fuerte.....	La Habra	Normal	0.08
Fuerte.....	La Habra	Normal	0.24
Fuerte.....	Montebello	Leaf-burn	0.15
Puebla.....	La Habra	Normal	0.15
Puebla.....	La Habra	Normal	0.29
Puebla.....	La Habra	Normal	0.24
Puebla.....	La Habra	Chlorotic	0.19

especially with overhead irrigation, so that no comparison can be made between the nitrogen content of burned and that of normal leaves. Flowers of Puebla trees at Riverside, California, were collected and were separated from the flower stalks. The total nitrogen of the flowers was found to be 1.71 per cent of the dry matter. At present it is not known whether failure of avocado trees to set fruit may be in any way related to the nitrogen content of the flowers.

A high nitrogen content of a soil may become of very great importance when the soil solution contains considerable amounts of chlorides and sulphates. HAAS and THOMAS (3) have shown the toxic effect of sulphates on lemon trees to be much greater when the nitrate supply was inadequate. As was previously mentioned, many of the coastal and other avocado districts are irrigated with water containing considerable chloride or sulphate. The occurrence of tip-

burn has not always been exclusively associated with the use of such irrigation water; in fact, it has been found to occur where the irrigation water was of good quality. The burning of a small portion of the leaf tip alone may occur as a consequence of an inadequate water supply, as was shown in sand cultures which received a culture solution containing no chlorine. In this case the sand cultures were covered but were not kept sufficiently moist, and although provided with a drainage system, they showed no drainage water except at the time of the addition of large amounts of new nutrient every three to

TABLE IV

NITROGEN CONTENT OF AVOCADO LEAVES

VARIETY	LOCALITY IN CALIFORNIA	CONDITION OF LEAVES	TOTAL N AS PERCENTAGE DRY MATTER
Fuerte.....	La Habra	Normal	2.52
Fuerte.....	Box Springs	Normal	1.87
Fuerte.....	Riverside	Normal	1.84
Fuerte.....	Riverside	Leaf-burn	1.80
Fuerte.....	Riverside	Leaf-burn	1.77
Fuerte.....	Culver City	Leaf-burn	1.76
Fuerte.....	Lemona	Leaf-burn	1.30
Taft.....	Riverside	Normal	1.98
Taft.....	Riverside	Leaf-burn	2.25
Taft.....	Lemona	Leaf-burn	2.20
Puebla.....	Riverside	Leaf-burn	2.07
Lyon.....	La Habra	Normal	2.53

four weeks. Such leaf-burn is not serious and is not the condition usually designated as tip-burn. In this latter condition one-fourth or more of the apical portion of the leaf may be brown and extremely desiccated, often with marginal burning proceeding farther toward the basal portion of the leaf. It may be mentioned that control cultures which were kept sufficiently moist were free from such tip-burn.

The causal factors producing this burned-leaf condition of the avocado tree, popularly called tip-burn, are best understood by comparing the analyses of normal avocado leaves (table V) with those of leaves affected with tip-burn (table VI). It is at once obvious that the leaves affected with tip-burn contain excessive amounts of total chlorine. In at least two of the cases examined, total sulphur may also be a contributing factor in bringing about

the burning of the leaves. It is of interest that in some cases very little sulphate was found in the leaves, even though the irrigation water contained a considerable amount.

TABLE V

TOTAL CHLORINE AND SULPHUR CONTENT OF MATURE, NORMAL AVOCADO LEAVES

VARIETY	COLLECTED	LOCALITY IN CALIFORNIA	PERCENTAGE IN DRY MATTER	
			Total Cl	Total S
Fuerte	3/16/26	Brentwood	0.33	.....
Fuerte	3/16/26	La Habra	0.32	.....
Fuerte	3/10/28	Riverside	0.09	0.25
Puebla	3/10/28	La Habra	0.21	.....
Taft	3/10/28	Riverside	0.17	0.29

TABLE VI

TOTAL CHLORINE AND SULPHUR CONTENT OF MATURE AVOCADO LEAVES SHOWING TIP-BURN

VARIETY	COLLECTED	LOCALITY IN CALIFORNIA	PERCENTAGE IN DRY MATTER		IRRIGATION WATER*	
			Total Cl	Total S	Cl	SO_4
					p. p. m.	p. p. m.
Fuerte	1/25/28	Culver City	0.91	.....	269–305	.....
Fuerte	3/24/26	Culver City	0.88	.....	269–305	.....
Fuerte	3/10/28	Riverside	0.91	0.31	195–213	140–254
Fuerte	3/10/28	Riverside	0.93	0.37	195–213	140–254
Fuerte	4/16/28	Montebello	1.00	.....	385	42
Fuerte	3/29/26	Montebello	1.21	0.88	.....	.....
Fuerte	3/29/26	Montebello	1.13	.....	.....	.....
Fuerte	4/13/28	Lemona	1.01	.....	52	.....
Fuerte	5/17/26	Irvine	0.80	.....	.....	.....
Seedling	3/16/26	Brentwood	1.34	1.13	.....	.....
Puebla	3/10/28	Riverside	1.01	0.22	195–213	140–254
Taft	3/10/28	Riverside	0.54	0.28	195–213	140–254
Taft	3/10/28	Lemona	0.72	0.28	52	28–32

* The writer is indebted to the Department of Chemistry of the Citrus Experiment Station for a portion of these analyses.

The chlorine content of the leaves of the trees at Lemona, California, is high in comparison with the low amount of chlorine in the irrigation water. In this case the water did not penetrate into the subsoil below the root zone. As root absorption and surface evaporation withdrew water from the soil, the chlorine that was added in the irrigation water could not escape into the subsoil, and conse-

quently accumulated in the moisture about the tree roots. The trees were given very little if any nitrogen, and as a consequence chlorine was absorbed in sufficient amounts to burn the leaves. In another of the coastal districts, in connection with studies on the growth of citrus trees, as conducted by FAWCETT and the writer, it was found that, although the irrigation water and the first two feet of soil were relatively free from chloride and sulphate, the third and fourth feet of soil were rather heavily charged with sulphate. It was found that chlorination of the irrigation water at the irrigation-district dam did not increase the chlorine content of the water upon its arrival at the groves.

A source of chlorine that makes its way into avocado groves is found in certain barnyard manures. In some districts the more saline water that is unsuited for tree culture is used in growing alfalfa, etc., for dairy purposes, with the result that such manure frequently finds its way into the groves. In a specific case the manure applied contained 23 pounds of chlorine in one ton of dried material. When this supply supplements an already somewhat saline irrigation water, the application may hasten the appearance of injury. In certain avocado groves where the trees were losing most of their leaves as a result of tip-burn, concentrations of chlorine (as determined from a one to five water extract of the soil) constituted one-third of the total solids obtained from such water extracts.

In the selection of sites which have been valued largely according to the degree of frost protection they afford, due consideration should be given also to the nature, depth, and drainage of the soil and subsoil, to the amounts of chloride and sulphate in the irrigation water, and to the nature of the materials used in the fertilization program.

Summary

1. The effect of lime-induced chlorosis on avocado trees is to raise the calcium-magnesium ratio.

2. The total and the water-soluble calcium of the dry matter of normal mature avocado leaves are considerably less than that of citrus leaves. The magnesium content of the dry matter of normal mature avocado leaves is two to three times that of normal mature citrus leaves. Chlorotic avocado leaves have been found to con-

tain large amounts of water-insoluble calcium and water-soluble potassium.

3. The total nitrogen and phosphorus contents of avocado leaves are approximately the same as those found in the leaves of citrus.

4. Tip-burn of avocado leaves has been found to be associated with a high chloride or sulphate content of the leaves.

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OOGENESIS AND FERTILIZATION IN VOLVOX

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 389

CAROLINE A. LANDER

(WITH PLATE XV)

Introduction

The recent taxonomic monograph of the Volvocales by PASCHER[1] suggested that a critical cytological study of some of the features in the life history might be worth while. A great deal of work has been done on *Volvox*, both by botanists and zoologists, but very little cytological work has been done with sectioned material.

Investigators have had difficulty in finding fertilization, and though it has been reported that sperms were seen in a boring motion, they have not been identified entering the egg. It is believed by some that they enter at the neck. No fusion stages have been reported. Although a study of fertilization was the chief object of this investigation, the invagination theory as presented by ZIMMERMANN[2] in 1925 was examined; and the formation of starch from the pyrenoids was traced through the life history.

ZIMMERMANN believes that as the asexual colony develops from the two to eight and on through the eleven successive divisions there is an arching, and gradually a hollow ball is formed. The pore enlarges and the colony turns inside out.

The formation of starch from the pyrenoids has been reported before in *Volvox*, and studied in detail in *Hydrodictyon* by TIMBERLAKE.[3]

Materials and methods

The material for this study was *Volvox globator*, collected in 1916 at Mud Lake, Ward, Colorado. It was killed in 0.5 per cent chromic

[1] PASCHER, A., Die Süsswasser-Flora. Volvocales. Jena: G. Fischer. 1927.

[2] ZIMMERMANN, W., Die Ungeschlechtliche Entwicklung von *Volvox*. Die Naturwissenschaften. Berlin. 13: 1925.

[3] TIMBERLAKE, H. G., Pyrenoid and starch formation in *Hydrodictyon*. Ann. Botany 15:619–635. 1901.

acetic acid and imbedded in paraffin. Sections were cut at 3 and 5 μ and stained by two methods. The safranin, gentian violet method was best for showing the formation of starch from the pyrenoid, the pyrenoid staining red and the starch blue. The sections were allowed to remain in the safranin over night, counterstained with gentian violet from one to ten minutes, according to the structures desired, and destained with clove oil. The other method used, Haidenhain's iron-alum haematoxylin, showed the nuclear structure better.

Investigation

Egg development

The cells of a colony are all apparently alike, each connected with its neighbor by protoplasmic strands, and containing a nucleus, an eye spot, and a chromatophore with a pyrenoid imbedded in it.

The first difference noticed in a reproductive primordium, or gonidium, whether asexual or sexual, is an increase in size (fig. 1). When a reproductive cell reaches about twice the diameter of a vegetative cell, the protoplasm has become more abundant and the sections stain more deeply. The outline becomes broadly pear-shaped or almost roundly triangular in cross-section. The cilia disappear before the increase in size takes place, or as it is taking place; it was not determined which. Even at this early stage the pyrenoid divides, so that such a cell contains two pyrenoids. From this stage the increase in size is very rapid, and is accompanied by an increase in the size of the nucleus and nucleolus, and a rapid increase in the number of pyrenoids (figs. 2, 3).

When the diameter has increased to six times the diameter of the original cell, the asexual gonidium and egg cell are indistinguishable; both are more broadly oval-shaped and contain six to twelve pyrenoids (fig. 3). From this point on, however, they differ somewhat. The nucleus of the egg moves to the center of the cell and enlarges (fig. 4). At this stage, the cell which is to form a new colony divides longitudinally, and thus is easily distinguished from one which is to form an egg.

After the nucleus of the egg has reached the center, its increase in diameter does not keep pace with the increase in protoplasmic content; but the pyrenoids continue to increase in number until the

egg is mature. The amount of food material increases up to the time of fertilization. At this time the egg has become rounded off within the parent cell, which is really an oogonium. At the time of fertilization it is a roundly pear-shaped body with a large nucleus containing a prominent nucleolus and rather scanty chromatin. Its cytoplasm is filled with various food materials, principally oil, volutin; and near the periphery, a zone of pyrenoids producing an abundance of starch, in the form of disk-shaped flakes surrounding each pyrenoid (fig. 5). The eggs of a colony seem to be of about the same size.

Invagination of daughter colony

Even after the first division of the asexual gonidium, in the two-celled stage there is an arching and the two cells seem to spread apart at the pore (fig. 6). This arching continues until in the eight-celled stage the axis is at right angles to the original axis (fig. 8). The nuclei remain toward the inside of the colony. This condition persists until eleven or more successive divisions have taken place, and then the colony turns inside out through the pore which has widened greatly (fig. 10). After this invagination, which causes the nuclei to be toward the outside, the cilia are formed.

Pyrenoid and starch

Starch is formed from the pyrenoids. Even in the vegetative cells a zone of starch is found around each one (figs. 1, 2, 3, 6, 7). In each stage of the division of the daughter colony (figs. 6, 7, 8, 9, 10), and all stages of the egg up through the mature zygote (figs. 1, 2, 3, 4, 5, 13, 17) slabs of starch are cut off. Some pyrenoids show just one piece of starch lying on its outer edge, but more show two, three, or even five or six pieces. These may form a definite zone around the pyrenoid, or they may finally come to lie in various positions near the pyrenoid (fig. 17). There may be a clear area between this zone and the edge of the pyrenoid, and the area sometimes takes a faint stain, as if starch were forming there in a dilute form. Sometimes two rings of starch are cut off around the pyrenoid. Because of the various pieces cut off the pyrenoids present peculiar shapes. The pyrenoid is most active in producing starch during the final

stages in the development of the egg before fertilization, and after fertilization until the zygote becomes vacuolate (figs. 4, 5, 13).

FERTILIZATION

The evidence shows that the sperm enters the egg from the interior of the colony. In many cases sperms were found lying on the oogonium wall on the side of the egg (fig. 11). One sperm was seen starting to penetrate the oogonium wall. The cilia could not be identified, and the nucleus was larger than those of the sperms in the mature sperm colony. The sperm enters, ciliated end first, through a pore in the oogonium wall (fig. 12). At the point of entrance there is a receptive spot or wider area between the cytoplasm of the egg and the outside of the oogonium wall (fig. 12). It appeared as if the cytoplasm of the egg had shrunk back to form the receptive spot, or some cytoplasm may have been discharged, as in *Oedogonium* and other algae.

As soon as the sperm enters, the border of the egg, which up to this time is merely a plasma membrane, begins to thicken and to stain like cellulose. For a while the sperm nucleus is hard to identify, probably on account of the dense mass of food material, for hundreds of eggs were examined at this stage, and the nucleus was not identified until it had almost reached the nucleus of the egg. The two nuclei come together with their nuclear membranes intact (fig. 13). Although the sperm nucleus has increased greatly in size since it entered the egg, it is slightly smaller than that of the egg, but their nucleoli are of about the same size. The membrane of the egg nucleus now becomes thinner at the point of contact, the membrane of the sperm nucleus breaks (fig. 14), and its nucleolus and the chromatin pass into the egg nucleus. The nucleoli remain separate (fig. 15), but the behavior of the two chromatin masses was not traced, except to note that in the resting zygote only one chromatin mass could be identified (fig. 17).

Discussion

This investigation, although brief, is sufficient to warrant the conclusion that ZIMMERMANN is right in his invagination theory. If the nuclei and chromatophores change places in the cell, as the older investigators reported, one ought to be able to find the nuclei in the

process of changing; but this I was unable to do. In all the divisions of the asexual colony up to the hundreds of cell stage, where there are too many to count, the nuclei are inward and no cilia have been formed. Mature colonies are found with the nuclei outward and the cilia present.

A number of stages identical with those of ZIMMERMANN were found (figs. 6, 7, 8, 9, 10). These may be due to the fixative, however, for the majority of the colonies, even the mature ones, take peculiar shapes caused by the fixation. On the other hand, ZIMMERMANN's photograph of the living colonies in the act of invagination is sufficient proof that this behavior occurs.

The starch formed from the pyrenoid has been noticed before in the Chicago laboratory in the egg stage; but it has not before been thoroughly traced through the life history or studied in detail. The present work confirms in *Volvox*, from the morphological standpoint, the work of TIMBERLAKE on the pyrenoid and starch formation in *Hydrodictyon*.

Other investigators are right in finding that fertilization is very difficult to identify. Hundreds of eggs were examined and only a few stages found. But without doubt the material showed many sperms closely applied to the oogonium wall, and in several cases the sperm nucleus was seen within the egg (figs. 13, 14). The pore for the entrance of the sperm and the receptive spot behind it were identified (figs. 11, 12). This does not support the suggestion of OLTMANNS[4] that the sperm enters at the neck of the egg. As yet no stages have been found from the time the sperm enters until the nucleus almost comes in contact with the egg nucleus. This present investigation shows the fusion stages (figs. 13, 14, 15, 16).

Summary

1. Starch is formed from the pyrenoids in the vegetative cells, in all stages of the division of the asexual colony, in all stages of the egg before and after fertilization, and in the mature zygote. It is most actively formed in the mature egg and in the early stages after fertilization.

[4] OLTMANNS, T., Morphologie und Biologie der Alden. II. Jena: G. Fischer. 1922-1923.

2. The nuclei of the daughter colony cells remain on the inward side of the cell, but as the colony matures the pore enlarges and the colony turns inside out through the enlarged pore.

3. Sperms enter the side of the egg from the inside of the colony through a pore in the oogonium wall, behind which is a receptive spot. The sperm nucleus increases greatly in size and comes in contact with the egg nucleus. The nuclear membranes of the egg and sperm dissolve at the point of contact, and the sperm nucleolus and its chromatin mass enter the egg nucleus. In the mature zygote the chromatin masses have united.

I am indebted to Professor CHARLES J. CHAMBERLAIN for suggesting the problem of fertilization of *Volvox*, which is the real aim of this investigation; and also to Professor W. J. G. LAND for valuable and kindly advice and encouragement during the progress of the work.

HOOD COLLEGE
FREDERICK, MD.

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EXPLANATION OF PLATE XV

Figures were drawn with camera lucida; reduced magnification ×856, except fig. 10, which is ×486.

FIGS. 1–3.—Very early stages of egg gonidium.

FIG. 4.—Young egg.

FIG. 5.—Mature egg at time of fertilization; *p*, pyrenoid surrounded by starch.

FIGS. 6–9.—Asexual colony, stages of development.

FIG. 10.—Asexual colony, beginning of invagination.

FIG. 11.—Mature egg and sperm (*s*).

FIG. 12.—Mature egg showing pore.

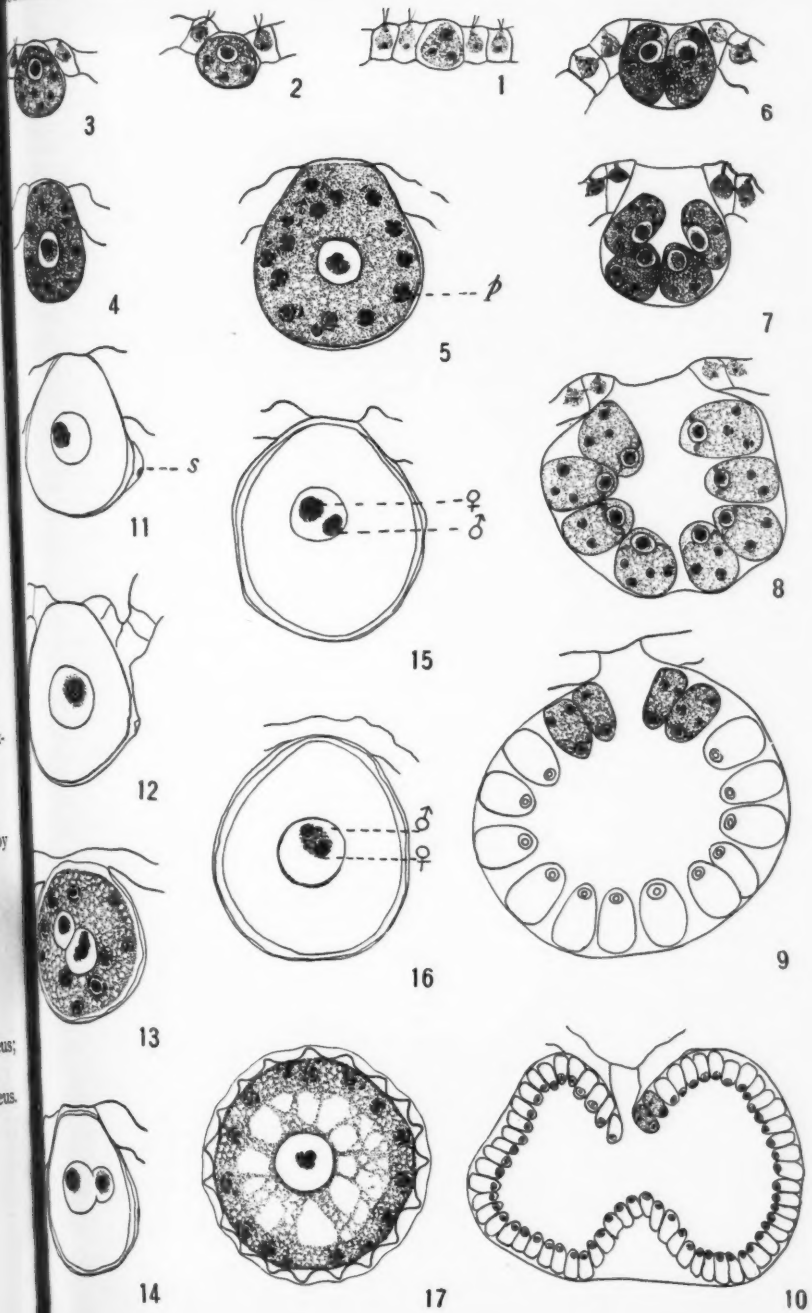
FIG. 13.—Fertilization, sperm and egg nuclei in contact.

FIG. 14.—Fertilization, wall broken between sperm and egg nuclei.

FIG. 15.—Fertilization, two nuclei and chromatin masses in egg nucleus; female nucleus, male nuclei.

FIG. 16.—Fertilization, two nuclei and one chromatin mass in egg nucleus.

FIG. 17.—Mature zygote.



LANDER on VOLVOX

CURRENT LITERATURE

BOOK REVIEWS

North American cup-fungi

For many years the study of the section Operculates of the order Pezizales (a section often referred to as the operculate cup-fungi) has been handicapped for American students by the absence of a monograph of that group. SEAVER has recently published a volume[1] which should remedy this situation. In his own words, it represents "a summary of our knowledge of the group which has accumulated after many years of more or less intermittent observation and study." Pages 3–35 are occupied with preliminary discussions, accompanied by illustrative figures, upon phylogeny, gross morphology, origin of the apothecium, ascospore formation, alternation of generations, heterothallism and homothallism, spore discharge, heliotropism or phototropism, dehiscence of the ascus, significance of the mode of dehiscence, eccentricity of the ascostome, asexual reproduction, ecology, pyrophilous cup-fungi, coprophilous cup-fungi, spore germination, viability of the spores, mycophagy, classification, geographical distribution, and nomenclature.

The Operculates are taken to consist of two families, namely, the Pezizaceae (by much the larger group, and including, for the range treated, 39 genera, among them the well known *Peziza*) and the Elvelaceae (including, for the range treated, only 5 genera, the best known of which to the average student is doubtless *Morchella*). Something of the great confusion that has arisen in the literature of the subject may be seen from the large number of synonyms and names of doubtful status that are listed. Thus, to take only a few examples, for the genus *Patella* (p. 156) 14 synonyms are given; for *P. adeboloides* (p. 170) 16 synonyms; for *Bulgaria melastoma* (p. 197) 16 synonyms; for *Morchella hybrida* (p. 241) 15 synonyms; and, as against 20 accepted species of Peziza (pp. 219–221), there are 301 names indexed as synonymous (a majority belonging to other genera) or of dubious significance.

As might be expected in a pioneer work of this type, the author has found it necessary to propose many new species and to introduce various new combinations. In all, some 366 species and 4 varieties are maintained. The descriptions are in English. These are followed by the type locality, distribution, list of illustrations in literature, and list of *exsiccati* in herbaria. Generally speaking, the so-called American Code is followed. There are numerous plates, most of them half-tones, and these are remarkable for their excellence.

[1] SEAVER, F. J., The North American cup-fungi (Operculates). 8vo. pp. vii+284. *pl. 45. figs. 15.* Published by the author, New York City. 1928.

Toward the end of the book an intimation is given that a second volume may eventually be published, to deal with the Inoperculates. It is to be hoped that such will be the case, and that it will conform to the same high standards attained throughout the present volume.—E. E. SHERFF.

NOTES FOR STUDENTS

Mitogenic rays.—In a recent issue of this journal[2] there appeared a review by R. O. EARL of a paper by GUTTENBERG criticizing the so-called "mitogenic ray" theory of GURWITSCH. It is perhaps proper to call attention to certain points in this review. It appears that both GUTTENBERG and EARL have based their criticisms, not on the original papers, but upon GURWITSCH'S recent review of his work,[3] which, it must be acknowledged, is badly written from the western point of view and often misleading.

In the first place, EARL says, presumably on the authority of GUTTENBERG, that "these rays are claimed to be emitted from actively dividing root-tip cells and to induce mitosis in neighboring roots." It should be pointed out that GURWITSCH has never made such a claim; on the contrary, his narcosis experiments have demonstrated that the supposed ray is emitted not from such cells but from the base of the root. According to GURWITSCH'S theory, a root does not emit these rays because it is dividing actively, but divides because the tissues (presumably leptome) at its base are emitting these rays. GURWITSCH considers the root tip the conductor but not the source of the ray. Moreover, he has never claimed that such a root would "induce mitosis in neighboring roots," unless the region of elongation of this neighboring root lies on a prolongation of the axis of the inducing root, a condition seldom met with in nature. Also, GURWITSCH and his co-workers have used as "sources" of the ray not only root tips of *Allium*, *Pisum*, and *Helianthus*, but also stem tips, cotyledons, and leaf vein tips of *Helianthus*, macerations of *Allium* bulbs, potato tuber leptome, yeast cells (both living and macerated), frog and axolotl eggs, the brains of tadpoles and axolotl embryos (both living and macerated), mixtures of haemoglobin from horse blood with serum from frog blood, arterial blood of frogs, etc., as well as the mercury vapor arc; and have used as detectors (induced organs) not only root tips of *Pisum* and *Allium* but also yeast cells, frog cornea, and other materials. To say that these rays emanate from root tips is true but misleading. GURWITSCH considers that they emanate from some embryonic tissues at periods of active metabolism (the emanation is suppressed under anaesthesia), but more especially from certain non-embryonic tissues. They can be produced in-vitro by mixing the extracted enzyme (mitotase), assumed to be a peroxidase, with some widely distributed oxidizable substance (mitotin), a behavior quite comparable with that of HARVEY'S "luciferase" and "luciferin," or to the materials

[2] BOT. GAZ. 86:119–120. 1928.

[3] GURWITSCH, A., Das Problem der Zellteilung physiologisch betrachtet. Berlin: Verlag Julius Springer. 1926.

apparently involved in the emanation of ultra-violet light from cod-liver oil. The use of potato tuber leptome as a source is especially interesting in view of its possible relation to the "leptome hormone" of HABERLANDT and LAMPRECHT. Both EARL and GUTTENBERG seem, if they have known at all of most of this work, to have ignored it.

Further on, after mentioning very briefly and uncritically the general features of GURWITSCH's technique, EARL continues, again apparently relying on GUTTENBERG:

> after at least twenty minutes the vertically placed tip (induced organ) was sectioned longitudinally, when many more mitoses were found on the side nearest the horizontal tip (inducing organ) than on that farthest from it. . . . . A point of note is made regarding median sections. Here many more cells are seen than in tangential sections and so more cases of division. Thus with higher totals greater deviations in number are to be expected.

No cognizance is taken of the two- to three-hour lag period required between exposure and the time of maximum observable effect as determined by GURWITSCH.

EARL also states:

> GUTTENBERG first points out the inadequacy of GURWITSCH's own data for a critical examination of the problem. The zones studied were not exactly indicated nor were the actual counts given in any complete or thorough manner. Figures were given in percentage of increase or numerical differences.

This is true of GURWITSCH's book, but not of the individual articles published from 1910 to date.[4] In most of these periodic papers, especially the earlier ones on which the theory was based, a careful record of actual counts on the "induced" and "non-induced" sides of the induced organ is given, and it is only in the briefer review that percentage differences alone are given. Moreover WAGNER, in verifying GURWITSCH's results, has insured against any possible error in determining the relative positions of induced and inducing root by fixing both roots *in situ*, imbedding in the same block, and sectioning them together. And further, GURWITSCH has controlled each series of experiments against a blank root exposed to a capillary tube filled with water and placed in the same relative position as the inducing root, another point which has apparently escaped his critics.

It seems strange that not one of those working with GURWITSCH (ANIKIN, BARON, FRANK, L. GURWITSCH, N. GURWITSCH, KISLIAK-STATKEWITSCH, RAWIN, RUSINOFF, SALKIND, or SORIN) has noticed the errors which seem so glaring to GUTTENBERG and EARL. The work has been verified in Prague under the direction of NĚMEC (WAGNER) and by MAGROU in France. It seems very un-

[4] Arch. Entwick. **30**:133–193. 1910; **32**:447–471. 1911; **52**:167–187. 1922; **100**: 11–40. 1924; **101**:53. 1924; **103**:68–79. 1924; **103**:490–498. 1924; **104**:109–115. 1925; **105**:470–472. 1925; **105**:473–474. 1925; **107**:829–832. 1926; **109**:362–. 1927, as well as numerous articles in the same journal by other workers.

fortunate that none of those who have criticized this work (EARL, LANDAUER, GUTTENBERG, and SCHWARZ), with the possible exception of the last named, has made any attempt to repeat it, using the GURWITSCH technique, or even to acquaint himself thoroughly with the theory he is attempting to combat. With this in mind, and considering the rapidly increasing mass of evidence tending to show that all "light," from the shorter radium rays to the short wave radio rays, has very important and as yet little understood effects on living tissues (and in many cases sources in living tissues), it seems to me that we should hesitate to say as yet that " 'mitogenic rays' are therefore due to join company with phlogiston, abiogenesis, 'n-rays,' and other discarded theories."—P. R. WHITE.[5]

Formaldehyde in photosynthesis.—Ever since it was proposed by VON BAEYER, over 60 years ago, the theory that formaldehyde is an intermediate product in photosynthesis has intrigued the fancy of plant physiologists; but all attempts to demonstrate that it is actually produced during that process have hitherto been either negative in result or open to invalidating criticism. Recently, however, KLEIN and WERNER[6] have published results of researches which seem to prove definitely that formaldehyde is produced during photosynthesis, and only then. By use of NEUBERG'S dimedon method with aquatic plants, it was possible to make determinations on living tissues, and formaldehyde was not only tied up *in vivo* as the stable aldomedon, but also diffused out into the environment almost quantitatively. Very careful account seems to have been taken of the possible sources of error, such as photolysis of sugars, chlorophyll, or organic acids, and assurance is given that no formaldehyde was detectable from these sources with the technique used. Especially convincing is the evidence obtained by use of WARBURG'S[7] technique for inhibiting photosynthesis by anaesthetics which are preferentially adsorbed. When small amounts of HCN or phenylurethane were added, production of formaldehyde ceased at once with cessation of O_2 output, whereas the continued formation of acetaldehyde showed respiration to be unaffected. And when the anaesthetic was removed and O_2 bubbles given off again, formaldehyde was formed once more. Likewise, when any single factor, such as light, CO_2, or temperature, was limiting, no formaldehyde was produced. It is unfortunate that pH measurements were not made, for no assurance is given that control experiments were run at the same alkalinity as the originals reach during photosynthesis. Like-

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[5] See also the review by GICKLHORN (Prague) in Protoplasma V. 4:625-628, 1929, of a book: REITER, T., and GABÓR, D., Zellteilung und Strahlung. pp. 183, *figs. 212*. Berlín: J. SPRINGER. 1928, defending on the basis of more than three years' careful experimentation, at the same time that it corrects in many places the work of GURWITSCH and his school, which appeared after the preparation of this note for publication.

[6] KLEIN, G., and WERNER, O., Formaldehyd als Zwischenprodukt bei der Kohlensäure-assimilation. Biochem. Zeitschr. **168**:361–381. 1926.

[7] Review in BOT. GAZ. **77**:345–346. 1924.

wise one wonders why illumination was always continued for at least 6 hours, when after 2 hours there is marked inhibition exercised by the reagent, or why 12 hours could yield more than 6 hours. But these criticisms do not at all invalidate the essential conclusions established.

More recently KLEIN and SVOLBA[8] have extended the work to some of the chemosynthetic bacteria, and have demonstrated that at least nitrite and thiosulphate bacteria produce formaldehyde as an intermediate step in their carbohydrate synthesis. They also found acetaldehyde formed during the respiration of these bacteria, as has previously been shown repeatedly for green plants.

In connection with the formation of formaldehyde during carbohydrate synthesis, the work of SABALITSCHKA is of interest. For many years he has studied the ability of plants to form starch and sugar from formaldehyde in the dark, but has left some loopholes for criticism each time. His last publication,[9] however, seems to offer valid proof that *Elodea* is able to polymerize formaldehyde to sugar and starch, in the absence of CO_2, in either light or dark, and more in dark than in light. The tissue was analyzed before and after the experiment for total hydrolyzable polysaccharides, and the test tissue found not only to contain more than the controls, but to have gained 4–12 per cent over the originals. Other workers,[10] following up this lead, have concluded that evidence from triturated leaf extract shows this polymerization to be due to enzyme action.—H. S. WOLFE.

Rate of photosynthesis.—An attempt to determine the rate of photosynthesis under approximately natural shady conditions has been made by MAXIMOW,[11] who measured the amount of CO_2 abstracted from the air by an attached leaf at successive intervals of time. The leaf was introduced into a flat glass vessel from which a rapid air current could be drawn, the air passing over the leaf so rapidly as to prevent any backward diffusion of CO_2. The size of the vessel (40 cc.), and rate of air flow (700 cc. per minute) through it, changed the air about the leaf nearly 18 times per minute. The air flowing by the leaf during 4–8 minute periods was analyzed for CO_2, and equal volumes of air were analyzed for controls. The amount of photosynthesis was calculated in terms of mg. CO_2 used during the brief period.

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[8] KLEIN, G., and SVOLBA, F., Zwischenprodukte bei Assimilation und Atmung autotropher Bakterien. Zeitschr. Bot. **19**:65–100. 1927.

[9] SABALITSCHKA, T., and WEIDLING, H., Über die Ernährung von Pflanzen mit Aldehyden. VI. Polymerisation des Formaldehyds durch *Elodea canadensis* zu höheren Kohlehydrate. Biochem. Zeitschr. **172**:45–57. 1926.

[10] BODNÁR, J., ROTH, L. E., and BERNAUER, C., I. Über die experimentellen Beweisen der Formaldehydassimilationshypothese. II. Die enzymatische Kondensation des Formaldehyds zu Zucker. Biochem. Zeitschr. **190**:304–325. 1928.

[11] MAXIMOW, N. A., and KRASNOSSELSKY-MAXIMOW, T. A., Schwankungen im Verlauf der Photosynthese. Ber. Deutsch. Bot. Ges. **46**:383–391. 1928.

The results showed very sudden and unexpected irregularities in the rate of synthesis, which MAXIMOW believes are natural variations in the speed of the reaction. A few of the results are given here. A buckwheat leaf in six successive 8-minute intervals used respectively 0.680, 0.016, 0.576, 1.032, 0.048, and 0.096 mg. of CO_2. The measurements for barley during eight 4-minute intervals were 0.792, 1.112, 0.964, 1.228, 1.114, 0.774, 0.912, and 1.114 mg., showing within about 40 minutes three rises and two decreases in rate. Similar results were obtained with millet and soy beans. Control determinations of the air itself did not vary over 3–5 per cent, while the leaves showed variations of 25–100 per cent or more.

Two possible causes are suggested for this periodic rise and fall of the rate. Over-accumulation of the synthate might depress the rate, and removal by diffusion might increase it. But more likely the periodicity is caused by periodic changes in the stomatal openings. BLAGOVESTSCHENSKI[12] and AGAMOV[13] have found that transpiration proceeds in the same fitful manner, and it is logical to assume that stomatal movement would affect the ingress of CO_2 in much the same way that it affects the egress of water vapor.—C. A. SHULL.

Fifth International Botanical Congress, Cambridge, England, 1930

NOTICE CONCERNING MOTIONS ON SUBJECT OF NOMENCLATURE

Motions on the subject of nomenclature for consideration by the Congress should be in the hand of the Rapporteur général, Dr. JOHN BRIQUET, before *September 30, 1929*.

Motions must be presented in the form of additional articles (or amendments) to the Rules of 1905–1910, drawn up in the form adopted in the *International Code*, and must be drafted as briefly as possible in Latin, English, French, German, or Italian. At least one hundred printed copies must be presented.

According to the decisions of the Brussels Congress, 1910, only motions relating to new points which were not settled in 1905 and 1910 can be presented. Motions which do not answer to these conditions shall only be discussed if the Cambridge Congress of 1930 decides to take them into consideration.

For further information about the programme of work for nomenclature, apply to the Rapporteur général, Dr. JOHN BRIQUET, Conservatoire botanique, Geneva, Switzerland.—F. T. BROOKS, Secretary.

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[12] BLAGOVESTSCHENSKI, A. W., The botanical station of the Central Asian University in summer. Bull. Univ. Asie Central, Taschkent. Lief. 7:8–14. 1924.

[13] AGAMOV, SARIBEK, Über die cuticularer Transpiration. Bull. Jard. Bot. Leningrad 26:576–594. 1927.